ANALYSIS OF HEAVY METAL MERCURY (Hg) WITH FISH IN THE CENTRAL KATINGAN RIVER USING ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

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Abstract

Central Katingan River is located in Central Kalimantan Province of Katingan Regency. The location of the river estuary is located in Katingan Kuala, where there are many illegal and legal gold mining that allows mercury heavy metal pollution (Hg) to exceed the normal limit on the body of fish which is an indicator of the occurrence of pollution in the water environment. Then the data is analyzed using qualitative and quantitative methods with the Atomic Absorption Spectrophotometer (AAS) instrument. This study aims to determine the presence or absence of heavy metal mercury (Hg) contained in fish in the Central Katingan River. This study also used analytical and cross sectional observation research methods using KI, HCl, and NaOH reagents and using of master solution ranging from 1000 ppm, 100 ppm, 10 ppm and using serial solutions of concentrations ranging from 2 ppm, 4 ppm, 6 ppm and 10 ppm. Based on the results of qualitative analysis, positive results were obtained, indicated by testing using KI and NaOH reagents, while the HCl reagent did not form a precipitate which showed negative results. Based on the results obtained in tilapia gill samples (A), mercury (Hg) levels of heavy metal were 0.140 mg/kg (A1), 0.129 mg/kg (A2), 0.022 mg/kg (A3), and carp gill samples (B).) levels of heavy metal mercury (Hg) were 0.138 mg/kg (B1), 0.132 mg/kg (B2), 0.046 mg/kg (B3). According to the Regulation of the Head of BPOM RI. Based on statistical analysis carried out using the SPSS application, it can be concluded that there is no significant difference in the levels of heavy metal mercury (Hg) in the two types of fish in the Central Katingan River, because the significance value <0.05.

Keywords: Fish, Mercury (Hg), Atomic Absorption Spectrophotometer (AAS)

Introduction

Indonesia is a maritime country that has a lot of oceans and rivers scattered throughout the region. One of them is the Katingan River, located in Central Kalimantan Province, Katingan Regency. The estuary of this river is located in Katingan Kuala, and this river has a length of 650 km and an average width of 250 m, and an average depth of 6 m. The Katingan River has two tributaries: the Kalanaman River on the left and the Samba River on the right. Katingan River is one of the largest and longest rivers in Central Kalimantan.

The Katingan River's people have enormous benefits, including a source of drinking water, a place for community activities such as bathing, washing dishes, washing clothes, agriculture, transportation, development of tourist areas, and a place for fish cultivation using cages. Another activity found in the Katingan River is illegal gold mining.

In cage fish cultivation, there are various types of fish, including tilapia and carp. This fish is very popular with local people because it tastes good and the price is also low. Therefore, traders in vegetable markets in Central Katingan also take and subscribe to caged fish cultivated by the local community. In the Central Katingan area, these fish are also traded in areas adjacent to Central Katingan. It is straightforward to breed people who cultivate these fish because this type of fish species is very adaptable to the environment in which they are raised.

The Central Katingan area has many illegal and legal gold mining. Gold mining activities are primarily found in the Katingan area, and this gold mining activity often triggers conservation and public health issues around gold mining (IFACS, 2014). Meanwhile, mercury compounds destroy the soil and as a separator for gold from impurities during illegal gold mining. The use of mercury compounds that are not by standard operating rules and for an extended period can have an impact on the biota in the Central Katingan River (Heriamariaty, 2012).

Considering the environmental conditions in the Central Katingan River allows heavy metal pollution of mercury (Hg) that exceeds the normal limit on the body of fish can be an indicator of pollution in the water environment. The threshold value of Hg metal set by WHO is 0 ppm, while according to the Regulation of the Head of BPOM RI No. 05 of 2018 (Maximum Limit of Heavy Metals in Processed Foods), the maximum limit of heavy metals in fish is 0.25 mg/day. kg (As), 0.20 mg/kg (except in processed predatory fish such as shrew, tuna, marlin 0.40) (Pb), 0.5 mg/kg (except in processed predatory fish such as shrew, tuna, marlin 1 0.0) (Hg), 0.10 (except in processed predatory fish such as shark, tuna, marlin 0.30) (Cd). The content of heavy metals mercury (Hg) in fish is closely related to waste disposal activities from gold mining around the river where the fish live (Rahman, 2016).

To analyze the presence of heavy metals in a compound, we can use qualitative and quantitative methods for quantitative research using the Atomic Absorption Spectrophotometer (AAS) instrument. The reason for using the AAS instrument is because the AAS instrument analyzes the concentration of heavy metals in the sample accurately. The concentration that is read on the AAS tool based on the amount of light absorbed which is directly proportional to the substance content and sample analysis at low levels, while other methods such as volumetric can only analyze at high levels, and the analysis carried out can take place quickly (Khopkar, 1990). Therefore, for heavy metal testing, it is most appropriate to use the AAS instrument.

In research conducted by Edison Harteman et al., December 2008, The content of heavy metals in the waters of the Katingan River positively contained Hg (0.0010 mg/L), Cd (0.0055 mg/L), and Pb (1.4263 mg/L) and for Katingan River sediments positively contained Hg (0.0125 mg/kg bb), Cd (0.0625 mg/kg bb) and Pb (5.2454 mg/kg bb), while heavy metals on hard fish fin bones positively contained Hg (0.0185 mg/kg bb), Cd (0.0295 mg/kg bb) and Pb (1.0637 mg/kg bb). Sediment increases along with the water concentration of the Kahayan River and Katingan River estuary areas. It explains the presence of heavy metal contamination in water and biota life in the Katingan River. One of the factors that allow becoming polluted is the existence of illegal gold mining activities located at the mouth of the Katingan River (Harteman, 2008).

Seeing from the various exposures that have been mentioned, the author is interested in examining the possibility of exposure and exposure to heavy metal mercury (Hg) in cultured Fish in the Central Katingan River area, which is quite close to the gold mining location. Therefore the author is interested in submitting a thesis with the title Analysis Heavy Metal Mercury (Hg) in Fish in the Central Katingan River Using Atomic Absorption Spectrophotometer (AAS).

Materials and Methods

The tools used in this research were AAS, stirring rod, porcelain cup, measuring flask, glass funnel, hot plate, scales, measuring flask, watch glass, beaker glass, Erlenmeyer, volume pipette, polyethylene bottle, measuring cup, dropper pipette, and spatula (SNI, 2011). The materials used in this research were the gills of caged fish taken in the Central Katingan River area, HgCl2, H2O2, concentrated HCl, concentrated HNO3, concentrated H2SO4, KMnO4, K2S2O8, NaOH, SnCl2, aquadest, Aluminum foil, and Whatman paper (SNI, 2011).

Wet Destructions

Weigh 10 grams of the sample, put the weighed sample into a 250 ml beaker, add 20 ml of nitric acid solvent p.a and 5 ml of pericolic acid p.a, and let the sample and solvent stand for one night. The dissolved sample was then heated on a hotplate at 90oC for 3.5 hours. See if the smoke turns white, remove it, and let it cool, then the sample is filtered using Whatman paper no.42. Save sample filtration. The sample is ready to be tested qualitatively and quantitatively (SNI, 2011).

Qualitative Analysis

1) Add 1 ml of the test solution, put it in a test tube, add five drops of KI solution. Observe the changes that occur, a positive result if it occurs in red-orange plants.
2) Add 1 ml of the test solution, put it in a test tube, add five drops of HCL solution. Observe the changes that occur. The results show a positive if there is a white deposit formation.
3) Add 1 ml of the test solution, put it in a test tube, then add five drops of NaOH solution. Observe the changes that occur. The results show positive if there is a yellow precipitate formation (SNI, 2011).

Quantitative Analysis

Manufacture of Master Solution for Hg (1000 ppm)

The manufacture of 1000 ppm of markuri master solution will require 100mg/100ppm. A total of 100 mg Hg was weighed using an analytical balance. After that, put it into a 100 ml volumetric flask and dilute the Hg with the addition of HNO_3 p.a to the mark of the volumetric flask (Permata Suka, 2020)

Manufacture of Raw Solution Hg (Mg/L) 100 ppm

Master solution 1000 ppm is pipetted 10 ml into a 100 ml volumetric flask, added with aqua dest to the limit of the mark, added with aqua dest flask to the mark, then the standard solution was pipetted 10 ml into a 100 ml volumetric flask to the mark limit.

Manufacture of Raw Solution 10 ppm

The 100 ppm master solution is picked 50 ml into a 50 ml volumetric flask, and then aqua dest is added to the limit mark, then the solution is shaken until homogeneous. A solution of 10 ppm is obtained.

Concentration Series Solution Making

Manufacture of a standard solution of 2 ppm; 4 ppm; 6 ppm; and 10 ppm As many as consecutively 20 ml, 40 ml, 60 ml, and 10 ml of the master solution 10 ppm will be inserted into a 100 ml volumetric flask using a pipette. Then add with HNO_3 to the volumetric flask with the aim of dilution (Permata Suka, 2020).

Sample Preparation

Weigh 10 grams of the sample, put the weighed sample into a 250 ml beaker, add 20 ml of nitric acid solvent p.a and 5 ml of pericolic acid p.a, and let the sample and solvent stand for one night. The dissolved sample was then heated on a hotplate at 90oC for 3.5 hours. See if the smoke turns white, removes, and calms, then filtered the sample using Whatman paper no. 42. Save sample filtration. The sample is ready to be tested qualitatively and quantitatively (SNI, 2011).

Measurement of Heavy Metal (Hg) Levels with Atomic Absorption Spectrophotometer

SSA instruments are optimized and prepared according to the instructions for use. Analysis of mercury levels in AAS uses the maximum wavelength. The instrument is first tested with a blank solution. After that, measure the absorbance of the Hg standard solution from low concentration to high concentration. Then take measurements on the sample solution resulting from wet digestion until the lead content is obtained (Permata Suka, 2020).

Results and Discussion

Results

a. Qualitative Analysis

The results of qualitative analysis that see in the form of color changes during processing where for the KI reagent there is a color change in the form of a brick-red precipitate, for the HCl reagent, there is a color change in the form of a white precipitate, and for the NaOH reagent, there is a color change in the form of a yellow precipitate.

The following is a qualitative test result of heavy metal mercury (Hg) :

Table 1. Qualitative test results of heavy metal mercury (Hg) in fish gill samples

Sample Code	Image	Treatment	Description	Result	Note
A1		Test solution added 5 drops of KI	Brick Red precipitate	+	Contains heavy metal Hg
A2		Test solution added 5 drops of HCl	White precipitate	-	There is no white precipitate
A3		The test solution added 5 drops of NaOH	Yellow precipitate	+	There is a yellow precipitate
B1		Test solution added 5 drops of KI	Brick Red precipitate	+	There is a brick red precipitate
B2		Test solution added 5 drops of HCl	White precipitate	-	There is no white precipitate
B3		The test solution added 5 drops of NaOH	Yellow precipitate	+	There is a yellow precipitate

Note: A1 = Sample of tilapia gills KI solution; A2 = Sample of tilapia gills HCl solution; A3 = Sample of tilapia gills NaOH solution; B1 = Sample of goldfish gills KI solution; B2 = Sample of goldfish gills HCl solution; B3 = Sample of goldfish gills NaOH . solution

b. Quantitative Analysis

Calibration Curve

The quantitative analysis begins with determining the standard calibration curve of mercury to obtain the linear equation y = bx + a. Calibration curves are obtained by measuring the absorbance of some concentrations of standard mercury sequence solutions using atomic absorption spectrophotometry with a mercury wavelength of 283.3 nm. The results obtained from the measurement of the mercury standard solution can be seen in table 4.3.

Table 2. Absorbance of mercury standard solution (Hg)

Standard Concentration (ppb)	Absorbance
2.0000	0.0018
4.0000	0.0336
6.0000	0.0616
10.0000	0.1330

(Source : Baristand,2021)

Based on the table above, make a standard curve for mercury (Hg) as shown below:

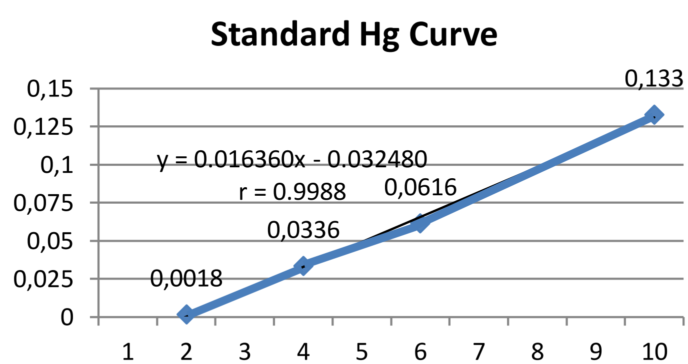

Figure 1. Relationship curve between concentration and absorbance of mercury standard solution (Hg)

Based on the calibration curve results above, the value of r (correlation coefficient) is obtained, namely mercury (Hg) 0.9988. The correlation coefficient (r) obtained from the standard mercury solution calibration curve can meet the line linearity requirements where 0.99 r 1 (Gandjar, 2007), so it can be concluded that the calibration curve obtained has good linearity.

With the linear regression equation for a standard solution of mercury, that is:

$$\boxed{Y = 0.016360x - 0.032480}$$

Note :

Y = Absorbance

X = Concentration

Slope = 0,016360

Intercept = 0,032480

Sample Quantitative Test Results

The following are the results of quantitative tests for heavy metal levels of mercury (Hg) in six samples of fish gills obtained from cages in the Central Katingan river.

Table 3. Quantitative test results of heavy metal mercury levels (Hg)

No	Sample	Absorbance	Concentration (ppb)	Mercury test results (Hg) mg/kg
1	A1	-0,0020	1,8631	0,140
2	A2	-0,0043	1,7225	0,129
3	A3	-0,0277	0,2922	0,022
4	B1	-0,0023	1,8447	0,138
5	B2	-0,0037	1,7592	0,132
6	B3	-0,0224	0,6161	0,046

Discussion

This research aims to find out whether or not there is a heavy metal content of mercury (Hg) in fish in the Central Katingan River, to find out the level of mercury heavy metal (Hg) in fish in the Central Katingan River, and to find out the difference in heavy metal mercury (Hg) levels in two different types of fish in the Central Katingan River. Analysis of heavy metals mercury (Hg) is analyzed on fish gills in the Central Katingan river using two methods: qualitative and quantitative analysis, then statistical data analysis. Qualitative analysis is carried out with KI, HCl, and NaOH reagents. Quantitative analysis using Atomic Absorption Spectrophotometer.

Qualitative Analysis

Qualitative analysis is a chemical analysis to determine the presence or absence of heavy metal mercury (Hg) in the sample. This test is carried out using 3 types of reagents, namely KI, HCl, and NaOH. The use of HCl as a reagent because HCl is a very commonly used reagent, and HCl can precipitate ions in it.

The test carried out is the heavy metal mercury (Hg) test. The sample test results by adding KI reagent to samples A and B showed positive results with the result that there was a red-brick precipitate. Heavy metal mercury (Hg) will react with I- ions from KI and form HgI2 (red brick precipitate). According to Vogel

(1990), the red brick precipitate will be lost by adding excess KI. Based on the results obtained, the positive sample contained heavy metal mercury (Hg) with the following reaction:

$Hg2^{+} + 2KI \longrightarrow (\downarrow)\ HgI_2 + 2K^{+}$

Qualitative testing of Hg metal is carried out by adding 5 drops of HCl to samples A and B. If a white precipitate formed, the result was positive. Based on the results obtained with the HCl reagent, the negative samples contained heavy metal mercury (Hg). Heavy metal mercury (Hg) in the sample will react with Cl- ions to form Hg¬2Cl2 (white precipitate) with the following reaction:

$Hg2^{+}_{(aq)} + 2Cl^{-}_{(aq)} \longrightarrow (\downarrow)\ Hg_2Cl_2$

Test by adding 5 drops of NaOH to samples A and B. If a yellow precipitate forms, it shows a positive result. Heavy metal mercury (Hg) in the sample will form Hg2O (yellow precipitate). Based on the results obtained with the NaOH reagent, the positive samples contained heavy metal mercury (Hg). These results are in agreement with the literature with the following reactions:

$Hg2^{+} + 2OH \longrightarrow (\downarrow)\ Hg + HgO + 2H_2O$

Positive results were indicated by testing using KI and NaOH reagents. The absence of a precipitate with HCl reagent can be caused by the level of heavy metal mercury (Hg), which is too low, so it is not detected during testing. In addition, the presence of disturbing factors can also cause no precipitate to occur when reacted. The disturbing factor can be in the form of impurities that are formed during the wet digestion process (Rahman, 2019).

Quantitative Analysis

The standard curve of Hg is made with five concentration series concentrations with an r-value of 0.9988. The value of the correlation coefficient (r) has met the requirements of the linearity test, namely 0.99 r 1 (Gandjar, 2007). Based on the results obtained in tilapia gill samples (A), mercury (Hg) levels of heavy metal were 0.140 mg/kg (A1), 0.129 mg/kg (A2), 0.022 mg/kg (A3), and carp gill samples (B).) levels of heavy metal mercury (Hg) were 0.138 mg/kg (B1), 0.132 mg/kg (B2), 0.046 mg/kg (B3). According to the Regulation of the Head of BPOM RI No. 05 of 2018 (Maximum Limit of Heavy Metals in Processed Food), the maximum limit of heavy metal mercury (Hg) in fish is 0.5 mg/Kg (except for processed predatory fish such as shrew, tuna, marlin 1 0.0 mg/kg). The level of heavy metal mercury (Hg) contained in the sample is still within the allowed range according to BPOM RI. Although the level of heavy metal mercury (Hg) is still safe, it is still necessary to pay attention so that the level does not exceed the maximum limit specified.

Several factors that cause heavy metal content in fish are coal loading and unloading activities, ship activities, public transportation, fishing boats (Cahyani et al., 2016), and gold mining activities (Harteman 2008). The level of heavy metal mercury (Hg) in the gills of the fish obtained was higher than the level of heavy metal mercury (Hg) in the water of the Central Katingan River. Research by Edison Harteman et al., 2008 stated that the level of heavy metal mercury (Hg) in the water of the Central Katingan River was 0.0010 mg/Kg. Higher levels of mercury (Hg) in fish gills can be caused by the accumulation of heavy metal mercury (Hg) in the fish body..

Statistic analysis

Normality test

A *normality test* is a statistical test used to assess the distribution of a group of data or variables. The Kolmogorov-Smirnov normality test is used if the number of data is > 30. Meanwhile, the Shapiro- Wilk normality test is used if the data is < 30.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Kadar_Ikan_Nila	.355	3	.	.819	3	.161
Kadar_Ikan_Mas	.364	3	.	.799	3	.111

a. Lilliefors Significance Correction

The data is normally distributed if the significance value is > 0.05. Based on the significance value obtained, it can be concluded that the data is normally distributed.

Homogeneity Test

Homogeneity test is a statistical test used to see whether or not the variances of two or more distributions are equal

Test of Homogeneity of Variances

Kadar

Levene Statistic	df1	df2	Sig.
.199	2	6	.825

Homogeneous data if the significance value > 0.05. Based on the significance value obtained, it can be concluded that the data is homogeneous.

Independent T Test

Independent T test is a statistical analysis used to compare the mean of two different and independent populations.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Kadar	Equal variances assumed	.408	.558	.174	4	.870	.008333	.047953	-.124804	.141471
	Equal variances not assumed			.174	3.796	.871	.008333	.047953	-.127676	.144342

Conclusion :

- If the significance value is < 0.05, then there is a significant difference in the gill samples of tilapia and carp levels in the Central Katingan River.
- If the significance value is > 0.05, then there is no significant difference in the gill samples of tilapia and carp levels in the Central Katingan River.

Conclusion

Based on the results of the analysis of the levels of heavy metal mercury (Hg) in the gill samples of Tilapia (*Oreochromis niloticus*) and Carp (*Cyprinus carpio*) qualitatively using KI, HCl, and NaOH reagents, it can be concluded that there is a heavy metal content of mercury (Hg) in the gill samples. Tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*) in KI and NaOH reagents and not detected in HCl reagent.

Based on the results obtained in tilapia gill samples (A), mercury (Hg) levels of heavy metal were 0.140 mg/kg (A1), 0.129 mg/kg (A2), 0.022 mg/kg (A3), and carp gill samples (B).) levels of heavy metal mercury (Hg) were 0.138 mg/kg (B1), 0.132 mg/kg (B2), 0.046 mg/kg (B3). According to the Regulation of the Head of BPOM RI No. 05 of 2018 (Maximum Limit of Heavy Metals in Processed Food), the maximum limit of heavy metal mercury (Hg) in fish is 0.5 mg/Kg (except for processed predatory fish such as shrew, tuna, marlin 1 0.0 mg/kg). The level of heavy metal mercury (Hg) contained in the sample is still within the allowed range according to BPOM RI. Based on the statistical analysis conducted using the SPSS application, it can be concluded that there is no significant difference in the levels of heavy metal mercury (Hg) in the two types of fish in the Central Katingan River because the significance value is <0.05.

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