

Effectiveness Test of Lime toward the Mercury Level Reduction in Nike Fish (*Awaousmelanocephalus*) In Gorontalo City

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Abstract

Gold mining activities can produce toxic mercury waste. Mercury can contaminate waters as well as fish which may cause health problems due to the accumulation of heavy metals in the body if it is consumed. Nike fish (*Awaousmelanocephalus*) is one of the fish species found in Gorontalo waters which is presumed to be endemic fish consumed by humans. This study aims to determine the effectiveness of lime juice in reducing levels of heavy metal mercury (Hg) in Nike fish. This study uses True Experimental research design by taking the population of Nike fish on the north coast of Leato, and the sample is Nike fish that are sold in the traditional market of Leato, Gorontalo City. Obtained data are analyzed by One-Way Analysis of Variance (ANOVA) Test and Post Hoc Test (LSD).

The results show that Nike fish that are soaked in 25% concentration of lime juice for 20 minutes, the concentration of mercury level decreases 0,02682 ppm, in 50% concentration, it decreases 0.02457 ppm, and in 75% concentration, it decreases 0.02140 ppm. Therefore, treatment of soaking Nike fish in lime juice can reduce the mercury level.

The conclusion is that lime juice is effective in reducing heavy metal mercury (Hg), and the higher the concentration, the lower the mercury content in Nike fish.

Keywords: Nike Fish; Lime; Heavy Metal Mercury (Hg).

1. Introduction

Nike fish is one of the fish species found in Gorontalo waters which is presumed to be an endemic fish and can be categorized as larva of *Awaous sp.* However, there have been no scientific studies either morphologically or genetically to determine the taxonomic status of Nike fish. Also, its bioecological studies have never been reported. ^{4,10}

Currently, the Nike fish has become a culinary attraction for tourists in Gorontalo area that mostly serves in fresh or cooked. This fish also becomes a regional export commodity that has high economic value. Soekanto et.al. states that frozen Nike fish has been exported to East Java

and become one of the products offered by an industry engaged in fish processing in the East Java region. This Nike fish (*Awaousmelanocephalus*) is a type of fish that is very popular to the community in Gorontalo.¹²

Awaousmelanocephalus or Nike fish usually appears only at the end of each month in the HijriCalendar (Islamic month) with a non-permanent fishing spot. Based on information obtained from fishing communities and field observations, there are two places for Nike (*Awaousmelanocephalus*) fishing spot in Gorontalo City, and it is the waters around Leato and the waters around TanjungKramat. Yet, the information about mercury contamination (Hg) in the two water areas is still not available.²

The existence of Unlicensed/Illegal Gold Mining (PETI) in the upstream watershed of the Bone River can cause these waters (Leato and TanjungKramat) to be polluted because these two areas are still affected by the flow of water from the Bone River.²

Water condition in the fishing area will certainly affect the biota includes Nike fish (*Awaousmelanocephalus*). The use of metal in Indonesia is increasingly widespread among the fields of household, education, arts, and especially in the field of industrial technology. Known that every aspect has its advantage and disadvantage, metal gives benefit to our daily work although it has a detrimental effect which can cause heavy metal poisoning.⁶

There are lots of people who do not realize that she/he has been exposed to metals on a daily basis, either in direct contact through air (breathing) or through digestion (food). Initially, a contaminated person does not give any sign due to the toxicity level in the body and are still tolerable yet accumulated. Metals that contaminate human body will not be excreted and will be accumulated, thus the poisoning symptom will occur when the level of toxin contamination has reached the normal threshold.⁷

Mercury (Hg) is one of the heavy metal contaminants, which is very important to note. It is because mercury may contaminate directly into natural waters from industrial waste disposal as well as through rainwater and soil washing.⁸

Mercury (Hg) is a dangerous heavy metal. We can find the excessive use of this metal as a chemical compound in laboratories and laboratory equipment. Organic compounds are also excessively used as pesticides, especially phenyl mercury *Dimethyldithiocarbamate*, which is used in paper mills, and ethyl mercuric chloride, which is used as a fungicide.¹⁵

The target of mercury poisoning (Hg) is nerve damage that can lead to paralysis and blindness. Based on the quality standards of Government Regulation No. 82 of 2001, the safe content of mercury (Hg) is 0.002 mg/ L.⁵

One of the activities that potentially leads to mercury (Hg) contamination in river and sea waters in the waters of Gorontalo Province is gold mining. Since 1940, mining and gold processing has been carried out by the Dutch government in Gorontalo Province.²

Mercury or quicksilver had been used in the gold extraction process. The process of how mercury contaminates rivers through mining activities changes the environment and causes more damage and loss than its benefits, especially the benefits for the communities around the mining area. Mining activities have polluted certain rivers such as the Taduluyunu River, Buladu River, Bone River, Il River, and Paguyaman River.¹⁶

Gold mining activities in Gorontalo Province consider as illegal due to the unlicensed or uncertified by government (PETI) that currently spread into several regencies in Gorontalo Province, one of those regencies is Bone Bolango Regency. The community processes the gold ore with mercury or quicksilver and cyanide. Thus, The liquid waste from the activity is discharged directly into a river near the mining area.²

The dangers of mercury (Hg) clinically affect the nerves such as (1) difficulty speaking or dysarthria, (2) blurred vision, (3) muscle weakness, cramps or movement disorders, and paralysis, (4) ringing or buzzing in the ears, and (5) impaired sense of taste both pain, touch or temperature, and shortness of breath. Mercury that contaminates pregnant or breastfeeding women will cause a severe effect on the development of the baby (motor impairment, mental function, hearing loss, and blindness) and even physical and mental disabilities.¹⁷

2. Materials and Method

2.1 Research Design

This research used a True Experimental research design because the samples have direct treatment to be soaked in lime juice with concentrations: 0%, 25%, 50%, and 75%. In this study, 4 treatments were conducted at each concentration, and five (5) repetitions were observed for 20 minutes to see the mercury level reduction. This study used (CRD) Completely Randomized Design Followed by sample repetition:

Table. Completely Randomized Sample Design

J1	K1	L2	M1
K2	J2	L1	M2
K5	J4	L4	M5
K4	J3	M3	L5
K3	M4	L3	J5

Note :

J = Lime 0% (Control)

K = Lime 25%

L = Lime 50%

M = Lime 75%

1,2,3,4,5 = Repetition

2.2 Research Variable

Independent variable: Lime juice with a concentration of 0%, 25%, 50%, and 75% within 20 minutes and the dependent variable: Mercury levels in Nike fish.

2.3 Population and Sample

The population and samples were Nike fish in the estuary of the Bone River, Gorontalo City. The samples of this study were 20 samples of Nike fish with 100 grams. For each concentration of lime juice, Nike fish used was 5 grams.

The sample criteria that were considered by researchers were Nike fish which were still fresh and had not been mixed with food preservatives.

3. Results And Discussion

3.1 Research Results

This research was conducted due to unwanted chemicals (mercury) in food and water because they can harm people. In this study, the mercury (Hg) test in Nike fish used the Atomic Absorption Spectrophotometric (AAS) method.

This study was conducted to test mercury (Hg) level reduction in Nike (*Awaous Melanocephalus*) by soaking Nike fish in the lime juice (*Citrus aurantifolia*) with various concentrations of 25% 50% and 75% with 5 repetitions within 20 minutes. The test was obtained by using 1 kg lime that is purchased at the central market of Gorontalo City. For pure lime juice, it is 42.5 ml

Nike fish used in this study was taken from Leato Utara Village, where the total weight of each sample was 100g. In conducting the treatment, the sample is soaked in lime juice for 20 minutes with 5 repetitions. The result of this treatment can be seen in the following table

Table 1: Results of Concentration Differences (0%, 25%, 50%, and 75%) to reduce mercury (Hg) level in Nike fish

Konsentrasi Larutan Jeruk Nipis (%)	Jumlah Sampel	Pengulangan	Hasil Pengujian (ppm)	Rata-Rata (ppm)	Metode Pengujian Dan Nilai Baku Mutu Rada Ikan Nike
0%	5 gram	I	0.02885	0,02884	SNI 01.2354.6-2006 (0,5 mg/kg)
	5 gram	II	0.02908		
	5 gram	III	0.02838		
	5 gram	IV	0.02909		
	5 gram	V	0.02880		
25%	5 gram	I	0.02738	0,02682	SNI 01.2354.6-2006 (0,5 mg/kg)
	5 gram	II	0.02709		
	5 gram	III	0.02504		
	5 gram	IV	0.02737		
	5 gram	V	0.02722		
50%	5 gram	I	0.02430	0,02457	SNI 01.2354.6-2006 (0,5 mg/kg)
	5 gram	II	0.02447		
	5 gram	III	0.02430		
	5 gram	IV	0.02429		
	5 gram	V	0.02548		
75%	5 gram	I	0.02104	0,02140	SNI 01.2354.6-2006 (0,5 mg/kg)
	5 gram	II	0.02119		
	5 gram	III	0.02167		
	5 gram	IV	0.02151		
	5 gram	V	0.02157		

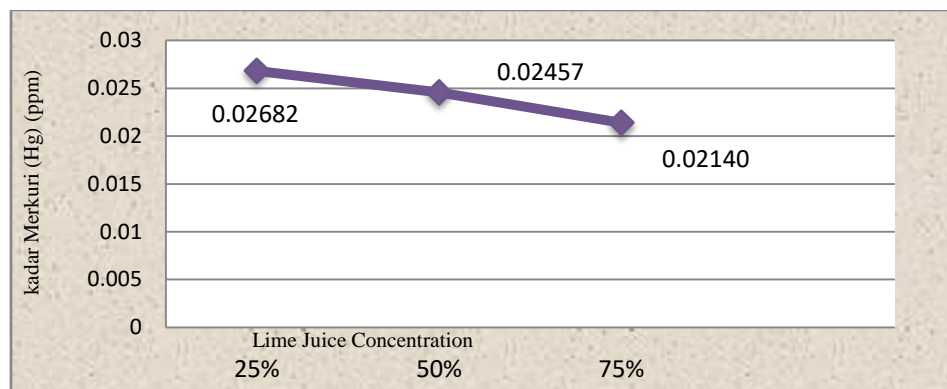
Source: Primary Data, 2020

Notes:

- *Konsentrasi Jeruk Nipis %* = Lime Juice Concentration
- *Jumlah Sampel* = Total of Samples
- *Pengulangan* = Repetition
- *Rata-rata (ppm)* = Average
- *Metode Pengujian Nilai Baku Mutu pada Ikan Nike* = Testing Method and Quality Standard Value in Nike Fish

In table 1, it can be seen that after the treatment that was observed for 20 minutes and at 5 repetitions showed that the highest average reduction in mercury (Hg) levels in Nike fish is at a concentration of 75%, with a result of 0.02140 ppm. The least mercury (Hg) reduction level in Nike fish is at a concentration of 25%, with a result 0.02884 ppm.

Figure 1 Graph of the correlation of the concentration of lime juice in reducing mercury (Hg) levels in Nike fish.



Source: Primary Data, 2020

Based on the test results in Table 1, a graph was made to describe the difference in the average amount of reduction in mercury (Hg) levels in Nike fish with various concentrations (0%, 25%, 50%, and 75%) of lime juice. From the graph above, it can be seen that the highest concentration (75%) has a significant influence in reducing mercury (Hg) level. Therefore, it can be concluded that the higher the concentration level of lime juice, the faster the mercury (Hg) levels will decrease in the Nike fish.

3.2 Data analysis

Data Normality Test

Before processing the data using One Way Anova test, the data obtained should meet the requirement that is normally distributed. By using SPSS program, Kolmogorov-Smirnov normality test was carried out. Based on the results of the normality test, it shows that the P value data on the mercury (Hg) reduction levels in Nike fish are explained by Asymp. Sig. (2-tailed), it is $0.610 > 0.05$ because the P value > 0.05 then H_0 is accepted, thus it can be concluded that the data are normally distributed.

3.3 Data Homogeneity Test

The requirement before using One Way Anova test is that the data must be normally distributed and have the same data variant or homogeneity. From the results of the variance homogeneity test using the *Levenetest*, it shows that the P value or Sig. is $0.131 > 0.05$ because the P value > 0.05 and H_0 is accepted, hence it can be concluded that the data hashomogeneous variants.

3.4 One-Way Analysis of Variance-ANOVA Test

Requirements for conducting one-way variance test or One Way Anova that is the data must be normally distributed and come from homogeneous variants. Based on the results above, the data arenormally distributed and have the same variants, hence it qualifies for the One Way Anova statistical test.

The results of the One Way Anova test show a significant value of 0.000 ($P < 0.05$), where H_0 is rejected and shows a significant difference between the treatment groups (using 25%, 50%, and 75% within 20 minutes) and control group in decreasing levels of mercury (Hg) in Nike fish. Therefore, it can be concluded that each concentration of lime juice can decrease the mercury (Hg) level in Nike fish.

3.5 Post Hoc Test (LSD)

LSD or the Least Significant Difference test is a follow-up test that is carried out if there is an effective difference. The Post Hoc test was conducted to determine the differences between treatments at each concentration. Based on the results of the tests, it shows ($P < 0.05$) in each

treatment. When viewed from the 25% concentration of the lime juice, it has a significant difference in the 50% concentration, and 75% in reducing mercury (Hg) levels both control group and treatment group.

4. Discussion

4.1 Lime effectiveness test to reduce mercury level (Hg) in Nike fish.

Based on the results of research in table 1 and in Figure 1, it shows that Nike fish which is originating from Leato Utara Village, Dumbo Raya Sub-district, Gorontalo City, are tested or analyzed at the BPPMDPP testing site (Institute for Testing the Application of Quality and Diversification of Fishery Products) Gorontalo City contains heavy metal Mercury (Hg), the level of 1st examination is: 0.02885 ppm, 2nd repetition is 0.02908 ppm, 3rd repetition is 0.02838 ppm, 4th repetition is 0.02909 ppm, and 5th repetition is 0.02880 ppm.

Levels of mercury (Hg) in Nike fish are still within safe limits for food because the limit of mercury (Hg) contamination which recommended by the food and drug regulatory agency No HK.00.061.1.52.4011, for levels of mercury (Hg) in Nike fish is 1, 0 ppm.³

However, if the Nike fish is consumed by humans continuously, there will be accumulation and destroy certain body organs, such as in the nerve which may cause difficulty speaking, blurred vision, muscle weakness, cramps or movement disorders and paralysis, ringing or buzzing in the ears and disturbances of taste both pain, touch or temperature, and shortness of breath. Mercury that contaminates pregnant or breastfeeding women will have a severe effect on the development of the baby (motor disorders, mental function, hearing loss, and blindness) and even physical and mental disabilities.

Based on the non-communicable diseases above, there are several non-communicable diseases that have a high prevalence and are generally often experienced by people with heart failure, bronchial asthma, hypertension, cervical cancer, diabetes mellitus, chronic kidney failure, eye disease or cataract, rheumatic disease, disease obesity, and mental illness.⁴

Seeing the health impacts caused by consuming Nike fish which contains heavy metal mercury (Hg), soaking the Nike fish using lime juice may help reduce mercury (Hg) levels in Nike fish. Lime which contains chemical compounds such as citric acid, can be a chelator agent or bind to metals contained in Nike fish.

Based on the results of mercury (Hg) level test that is soaked in lime juice, there is a significant decrease of mercury (Hg) which contains in Nike fish. The ability of lime juice to reduce mercury (Hg) levels in Nike fish is due to the presence of citric acid (COHO). The OH and COOH functional groups in citric acid create metal ions to form citrate salts. Citrate ions will bind the metals through a chelating process so that they can remove metal ions that have accumulated in Nike fish as citrate complexes.⁸

Besides citric acid, it also contains ascorbic acid. Ascorbic acid will cause complex bonds with metal ions. Ascorbic acid has one free electron pair of its molecule, which can be assigned to metal atoms which create Hg-ascorbic acid.⁹

According to WHO (World Health Organization), if the fish is continuously consumed as much as 60 g/ person/day, then the maximum Hg level allowed is 0.5 µg/ g of wet fish¹¹

Based on WHO standards in (Simage, 2010) regarding the number of mercury levels that can be accumulated in the human body for one week is 0.3 ppm, total mercury per week is 0.2 ppm, and per 70 kg body weight is 0.04 ppm per day.

Research has been carried out on Marcia Hiantina shells that had originated from Jl. Metro Tanjung Bungan by taking samples twice, on April 12, 2015 and April 16, 2015. Further, it soaked in lime juice with 15% concentration with variations of soaking time of 30 and 60 minutes and also used distilled water (*Aquadest*) with the same time variation in the sample as control. Based on the treatment, the Pb level on the first treatment was 0.5265 (µg / g), and the second treatment was 0.4308 (µg / g). After soaking treatment, there was a decrease in Pb level in the shellfish meat, both soaked using distilled water (*Aquadest*) for 30 and 60 minutes and with lime juice with the same time variation (30 and 60 minutes).¹

Two samples were soaked with distilled water (*Aquadest*), both samples decreased with an average decrease is 16.63% for 30 minutes and 20.08% for 60 minutes. Meanwhile, the samples soaked with lime juice obtained a significant score of the decreasing level compared to distilled water, the reduction level was 45.74% for 30 minutes and 64.37% for 60 minutes.¹⁴

In terms of the texture of the shellfish, it did not change before and after soaking. In the taste aspect, color and smell, there were differences before, and after soaking, a long duration of soaking caused a sour taste, reduce the fishy smell, and created paler meat. The best results can be seen in soaking with lime juice for 30 minutes because it does not change the taste, color, and texture but can eliminate the fishy smell.

4.2 Concentrations differences (0%, 25%, 50%, and 75%) of mercury (Hg) level reduction in Nike fish

The results of Table 1 shows a significant decrease level of mercury (Hg) after the treatment of soaking Nike fish in lime juice with various concentration for 20 minutes. It can be seen that the highest levels of mercury (Hg) are found at a concentration of 0% in 20 minutes observation. In contrast, the lowest mercury (Hg) levels in Nike fish were found in 75% concentration of lime juice within 20 minutes of observation.

Figure 1 shows the average percentage of mercury (Hg) levels in Nike fish at various concentrations (25%, 50%, and 75%). In 25% concentration of lime juice within 20 minutes observation toward Nike fish, the average decrease is 0.02682 ppm, at a concentration of 50% is 0.02457 ppm, and at a concentration of 75% is 0.02140. The highest concentration is 75% and causes a higher decreasing level of mercury (Hg) in Nike fish. Hence, it can be concluded that the higher the concentration of lime juice is used, the higher result of mercury (Hg) level reduction in Nike fish.

Balawan waters are polluted by dangerous and poisonous heavy metals. This is due to the presence of several industries, which are the main contributors to heavy metal pollutants in the Deli river (Hayati, 2009). According to research by Bapedalda North Sumatra in 2013, the content of mercury (Hg) in Belawan Sea reached 0.7012 mg/ L. whereas, based on the quality standards of government regulation No. 82 of 2001, the safe content of mercury (Hg) is 0.002 mg/ L.¹³

Tuna test was conducted on April 14, 2015, and the tested samples were taken from Medan Regional Health Laboratory. The tested samples showed that the mercury (Hg) level in Tuna which originated from Belawan was 0.0875 ppm with the AAS method. The results of the laboratory test showed that the mercury level in tuna fish before soaked with lime juice was 0.0875 ppm. This score is still below the average of the quality standard set by the Head of BPOM Number HK.00.06.1.52.4011 in 2010, which concerns the determination of the maximum limit of microbial and chemical contamination in food that processed fish has a maximum limit of mercury content which is 0.5 mg/kg³.

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5. Conclusion

1. Lime juice (*Citrus Aurantifolia*) is effective in reducing levels of mercury (Hg) in Nike fish (*Awaousmelanocephalus*) with various concentrations within 20 minutes. Based on the One Way Anova test, it shows a significant value of 0.000 (p-value <0.05), then H_0 is rejected thus, the lime juice has an effect on reducing mercury (Hg) levels in Nike fish.
2. The most significant concentration of lime (*Citrus aurantifolia*), which can reduce mercury (Hg) levels in Nike fish (*Awaousmelanocephalus*) is at a concentration of 75%.

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