Bioaccumulation of Copper (Cu) and Chromium (Cr) on export commodity vanamei shrimp from Karawang, West Java

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Abstract. Karawang is one of regencies in West Java which has great potential for vannamei culture. The farm here was modern farm and using Citarum River as water source. Human activities like household and industry around the river cause its quality decrease and give negative impact to shrimp farm. This research was aimed to investigate the bioaccumulation of copper (Cu) and chromium (Cr) on vannamei shrimp from Karawang, West Java. Amount of shrimp’s meat and carapace were used for heavy metal measurement using Atomic Absorption Spectrophotometry. Result showed that contents of copper both in meat and carapace were higher than content of chromium. Moreover, the content of both metals was higher on carapace compared to meat. Since the content of meat were below threshold, so it is safe for consumption. There is no significant difference content of both metals in carapace.

1. Introduction

Shrimp is one of the main commodities of Indonesian aquaculture, so it is expected to be able to support the country's economy fromo the fishery sector. In addition to cultivation technology, it has been known that this shrimp has on both domestic and foreign markets and became a major factor in the increasing growth of shrimp farming in Indonesia. Based on the statistical data of aquaculture fishery in 2015, it is stated that the average increase of shrimp farming production volume of Indonesia from 2011 to 2015 amounted to 13.43% per year. Karawang regency is one area in West Java Province, which has great potential for the development of vanamei shrimp farming. Potency of pond area owned by Karawang regency is equal to 18.275 ha, and only used for 15.828.80 ha or equal to 86.61% with amount of production of shrimp produced to reach 11.756,61 ton [1].

Decreasing quality of Citarum River is very alarming, it is known that along 127 km or 47.1% of the length of river has been categorized as heavily polluted [2]. The existence of several industries around the Citarum River Basin (DAS) as well as the entrance to several tributaries along the Citarum River gives effect on presence of heavy metals especially Copper (Cu) and Chromium (Cr) in the waters of the Citarum River [3] which implies the declining quality water in aquaculture area. Water sources that indicated by contamination of heavy metals will potentially affect the quality of pond water so that it
will have implications on the decline of productivity and quality of vaname shrimp produced in Karawang regency West Java Province.

Fulfillment of water quality for cultivation in accordance with the needs of living vaname shrimp is a key for the success of the cultivation business. Small amount of heavy metal content in water, can be absorbed and biologically accumulated in shrimp maintained in ponds. Contamination of heavy metals on shrimp tissue or body will disrupt organ function of shrimp body or even death, causing decrease of production level of cultivated shrimp production. If the shrimp is consumed by humans for long periods of time then it may pose a risk for health. Water quality in ponds not only determine the success of shrimp farming but also related to quality assurance and safety on cultivated fishery products.

In addition to domestic or local consumption, shrimp is Indonesia's leading fishery export commodity. Directorate General of Competitiveness Improvement, Ministry of Marine Affairs and Fisheries stated that shrimp exports Indonesia experienced an average increase of 8.7% per year [4]. Shrimp carapace is a solid waste of economic value that can be sold as export waste. Shrimp production generates waste ± 35% -50% of shrimp weight. Most shrimp processing businesses produced waste head, skin and tail. Shrimp skin contains protein (25% - 40%), chitin (15% -20%) and calcium carbonate (45% -50%) [5]. Exports are made in large quantities to ship to China. China is a country that is currently able to process shrimp waste for recycling [6].

Research on the content of heavy metal residues of copper (Cu) and chromium (Cr) on shrimp vaname (Litopenaeus vanamei) cultivation in Karawang West Java Province has been done to find out how is the impact of heavy metal pollution on the cultivation of vaname shrimp and to compare between the concentration of heavy metal copper (Cu) and chromium (Cr) in meat and carapace.

2. Methods

2.1. Research Location and Time Sampling
The sampling was conducted at Balai Perikanan Perikanan Budidaya (BLU-PPB) Karawang, located in Pusakajaya Utara Village RT 04 / RW 01, Cilebar District, Karawang Regency, West Java Province in April 2017 period.

2.2. Shrimp Sampling
Vaname Vaname shrimp (Litopenaeus vannamei) was cultivated in six maintenance plots. Sampling was done as much as two retrials (age 40 and age 55 days) at random (random sampling) as many as 30 individuals in each plot that has been determined. The weight of the shrimp was weighed and separated out the meat and the carapace. The sample preparation for heavy metal content analysis refers to [7].

2.3. Heavy Metal Measurement
Copper (Cu) and chromium (Cr) was analyzed at the Chemical Services Laboratory, Department of Chemistry, FMIPA, University of Indonesia using Atomic Absorption Spectrophotometer AAS Shimadzu series 6300. Copper and cadmium testing refers to [7].

2.4. Data Analysis
The data analyzed were age data, shrimp weight and heavy metal content of Cu and Cr both in meat and in carapace. Data analysis method used was Multivariate Analysis Of Variance (MANOVA) calculated using SPSS 24 (windows) to know the effect of age and weight of shrimp on heavy metal content of Cu and Cr in meat and in carapace.

3. Result and Discussion
Table 1 illustrated the data of Copper (Cu) and Chromium (Cr) test results on meat and carapace grouped by the sampling time. Figure 1 shows the average weight, copper content (Cu) and Chromium (Cr) on meat and carapace which were grouped according sampling time.
Table 1. Content of copper and chromium in meat and carapace of vanamei shrimp

|          | First sampling |          | Second sampling |          |
|----------|----------------|----------|-----------------|----------|
|          | Meat           | Carapace | Meat            | Carapace |
|          | Cu             | Cr       | Cu              | Cr       |
| 1        | 5.42           | 0.55     | 3.62            | 0.34     |
| 2        | 5.49           | 0.45     | 3.63            | 0.36     |
| 3        | 5.51           | 0.60     | 5.89            | 0.32     |
| 4        | 3.13           | 0.85     | 2.93            | 0.26     |
| 5        | 2.93           | 0.42     | 2.39            | 0.30     |
| 6        | 2.42           | 0.47     | 2.37            | 0.45     |

Table 1 showed that the data of copper (Cu) test for the lowest shrimp meat is 1.94 ppm and the highest are 5.51 ppm. While the results of copper (Cu) analysis showed that the lowest carapace was 2.37 ppm and the highest were 5.89 ppm. According to the average value of copper content (Cu), it seems that the content of copper (Cu) in shrimp meat is greater than the contents of copper (Cu) on the carapace. The decline in the value of copper content (Cu) in the meat on second sampling was 0.60 ppm when compared to the second sampling on the carapace, copper content (Cu) was 0.43 ppm when compared to second sampling. The lowest chromium (Cr) contents in meat was 0.04 ppm and highest was 0.85 ppm. While in carapace, the lowest value of chromium (Cr) was 0.26 ppm and the highest are 0.83 ppm. Results of chromium (Cr) analysis showed that in the meat on second sampling, there was a decrease in the value of chromium (Cr) which was 0.32 ppm, whereas in the carapace there was an increase of 0.14 ppm.

Heavy metal is a high molecular weight element. In low levels, heavy metals are generally toxic to plants and animals, including humans. Heavy metals that often contaminate the habitat is Hg, Cr, Cd, As, and Pb [8]. Heavy metals enter into the waters, either rivers or seas will undergo such processes as deposition, adsorption and absorption by aquatic organisms and will enter or into the shrimp body through metabolic processes.

Heavy metals become dangerous due to the bioaccumulation system, which increases the concentration of chemical elements in living organisms [9]. According to [10], the factors that cause heavy metals to be included in the pollutant group are due to the properties of heavy metals that are non-degradable and easily absorbed.

The normality test of dependent data is done by looking at the distribution of QQ Plots. MANOVA to see the most appropriate data normality is to use Q-Q Plots. Because of using this analysis can be seen as a whole the dependent variable is normal or not normal distribution. The results of the analysis of normality test based on the distribution of Q-Q Plots can be seen in Table 2 and Figure 1.

Table 2. Estimated Distribution Parameters

|                  | Cu-content | Weight | Cr-content |
|------------------|------------|--------|------------|
| Normal Distribution | Location   | 3.5433 | 6.5192     | .4025      |
|                  | Scale      | 1.23767| .88745     | .18595     |

The cases are unweighted.

From Figure 1, it can be seen that the distribution of Cu, Cr content and weight is not normal distribution, this is caused by the dot distribution is not on linear line (spread).
The equality matrix test of covariance variance can be done by using Box’s M Test test. The results of the analysis of equality test matrix variance-covariance can be seen in Table 3.

### Table 3. Box’s test of equality of covariance matrices

|       | Box’s M | F   | df1  | df2   | Sig.  |
|-------|---------|-----|------|-------|-------|
|       | 11.926  | .475| 18   | 1413.497 | .969  |

Value of p value> 0.05, so the variable of Cu, Cr content and weight have variance-covariance matrix which is not different, or it can be concluded that dependent variable does not fulfill equality of variance-covariance matrix.

In MANOVA there are several test statistics that can be used to make decisions in group differences, such as Pillai’s Trace, Wilk’s Lambda, Hotelling’s Trace, and Roy’s Largest Root. However, the test statistic used only Pillai’s Trace. This is because of the unfulfilled assumptions such as the assumption of normality and the assumption of equality of the opposite variance-covariance matrix. The results of multivariate test analysis can be seen in Table 4 below.

### Table 4. Multivariate tests

| Effect       | Value | F     | Hypothesis df | Error df | Sig. | Partial Eta Squared |
|--------------|-------|-------|---------------|----------|------|---------------------|
| Intercept    | .990  | 605.354 | 3.000        | 18.000   | .000 | .990                |
| Organ        | .067  | .428  | 3.000        | 18.000   | .735 | .067                |
| Age          | .529  | 6.746  | 3.000        | 18.000   | .003 | .529                |
| Organ * Age  | .424  | 4.421  | 3.000        | 18.000   | .017 | .424                |

a. Design: Intercept + Organ + Umur + Organ * Umur
b. Exact statistic

From Table 4, it can be seen that in the organ variable the significance value tested in the Pillai’s Trace procedure shows a value> 0.05. This indicates that the variable Cu, Cr content and weight together stated no significant difference in the meat and carapace organ. In the age variable, the significance values tested in the Pillai’s Trace procedure show <0.05. This means that the variables of Cu, Cr and Cr content together express significant differences in shrimp life.
Levene's test test is intended to see the homogeneity of each variable. From result of Levene's test of Equality of Error Variances seen that weight variable, Cr content and Cu content value > 0.05 so it can be concluded that those variables do not fulfill the assumption of homogeneity (p < 0.05).

Table 5. Levene's test of equality of error variances

| Variable   | F    | df1 | df2 | Sig. |
|------------|------|-----|-----|------|
| Weight     | .000 | 3   | 20  | 1.000|
| Cr_content | 1.126| 3   | 20  | .362 |
| Cu_content | 1.475| 3   | 20  | .252 |

Main effect is used to test whether there are significant effects based on organ types on shrimp weight, Cu and Cr levels individually. And whether there are significant effects on shrimp age, shrimp weight, Cu and Cr levels individually. The results of the analysis of the significance of each independent variable to the dependent variable can be seen in the Table 6.

Table 6. Tests of between-subjects effects

| Source         | Dependent Variable | Type III Sum of Squares | dff | Mean Square | F      | Sig. | Partial Eta Squared |
|----------------|--------------------|-------------------------|-----|-------------|--------|------|---------------------|
| Corrected Model| Weight             | 7.107^a                  | 3   | 2.369       | 4.304  | .017 | .392                |
|                | Cr-Content         | .362^b                   | 3   | .121        | 5.571  | .006 | .455                |
|                | Cu-Content         | 3.728^c                  | 3   | 1.243       | .789   | .514 | .106                |
| Intercept      | Weight             | 1019.989                 | 1   | 1019.989    | 1853.284| .000 | .989                |
|                | Cr-Content         | 3.888                    | 1   | 3.888       | 179.494| .000 | .900                |
|                | Cu-Content         | 301.325                  | 1   | 301.325     | 191.292| .000 | .905                |
| Organ          | Weight             | .000                     | 1   | .000        | .000   | 1.000| .000                |
|                | Cr-Content         | .001                     | 1   | .001        | .028   | .869 | .001                |
|                | Cu-Content         | 1.938                    | 1   | 1.938       | 1.230  | .281 | .058                |
| Age            | Weight             | 7.107                    | 1   | 7.107       | 12.913 | .002 | .392                |
|                | Cr-Content         | .049                     | 1   | .049        | 2.244  | .150 | .101                |
|                | Cu-Content         | 1.717                    | 1   | 1.717       | 1.909  | .309 | .052                |
| Organ * Age    | Weight             | .000                     | 1   | .000        | .000   | 1.000| .000                |
|                | Cr-Content         | .313                     | 1   | .313        | 14.441 | .001 | .419                |
|                | Cu-Content         | .073                     | 1   | .073        | .046   | .832 | .002                |
| Error          | Weight             | 11.007                   | 20  | .550        |        |      |                     |
|                | Cr-Content         | .433                     | 20  | .022        |        |      |                     |
|                | Cu-Content         | 31.504                   | 20  | 1.575       |        |      |                     |
| Total          | Weight             | 1038.103                 | 24  |             |        |      |                     |
|                | Cr-Content         | 4.683                    | 24  |             |        |      |                     |
|                | Cu-Content         | 336.557                  | 24  |             |        |      |                     |
| Corrected Total| Weight             | 18.114                   | 23  |             |        |      |                     |
|                | Cr-Content         | .795                      | 23  |             |        |      |                     |
|                | Cu-Content         | 35.232                   | 23  |             |        |      |                     |

a. R Squared = .392 (Adjusted R Squared = .301)  
b. R Squared = .455 (Adjusted R Squared = .374)  
c. R Squared = .106 (Adjusted R Squared = -.028)

From Table 6, it can be seen that the corrected model, the influence of the independent variables (organ and age, and the interaction of organ * age together to the dependent variable (weight, Cu and Cr levels not significant with sig value > 0.05. Dependent (weight, Cu and Cr concentration) without
influenced by independent variable (organ and age) is worth sig <0.05 it means that dependent variable (weight, Cu and Cr content) can change its value without independent variable influenced that organ and age Significant intercept). The influence of organs on weight, Cu and Cr levels respectively valued > 0.05 this means that the organs have no effect on weight, Cu and Cr content. The influence of age to weight has a probability value or sig <0.05, Shrimp will affect shrimp weight. While the influence of age to Cu and Cr has a probability value or sig > 0.05 which means that age variable does not affect Cu content of Cu and Cr in the body of shrimp. The result of interaction between organ * age to weight and Cu value is > 0.05 or not significant, however interaction result between age organ against Cr content is <0.05 which means significant or interaction between age * organ affect Cr content.

4. Conclusion
Content of copper and chromium on this shrimp were below threshold, which indicated that shrimp still safe for consumption. Content of copper and chromium in carapace were significantly similar which possessed similar risk if it is used for industrial need.

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