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Exposure copper heavy metal (Cu) on freshwater mussel (*Anodonta woodiana*) and its relation to Cu and protein content in the body shell

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Abstract. *Kurnia AI, Purwanto E, Mahajoeno E. 2010. Exposure copper heavy metal (Cu) on freshwater mussel (*Anodonta woodiana*) and its relation to Cu and protein content in the body shell. Nusantara Bioscience 2: 48-53.* To determine the relationship of Cu exposure in water to the freshwater mussel exposure experiment is conducted with water containing Cu. Which measured the influence of Cu and protein content in the body shell. This study used the freshwater mussel species, *Anodonta woodiana*. Oysters were exposed for four weeks in the water with Cu concentration of 0.02 ppm, 0.04 ppm, 0.06 ppm, and 0.00 ppm control. Cu content and protein content in the body shells are checked every week. Cu analysis was done by Atomic absorption spectroscopy (AAS) method and the protein content using Kjeldahl method. Cu analysis showed elevated levels of Cu in mussel body after exposure. The pattern of increase in Cu content was not the same, where the pattern of the largest increases occurred after the fourth week. The statistical test showed no significant effect between the treatment with Cu accumulation in the body shell. Protein analysis showed an increase of protein content after exposure of the second week and decreased after the third and fourth weeks. The pattern of changes in protein content varied among the various treatments. The statistical test showed no significant effect between treatment with the protein changes in the body shell. Correlation test of the relationship between concentration of Cu in mussel body protein level showed a positive correlation between them with a fairly good level of relationship (correlation coefficient r = 0.836).

Keywords: *Anodonta woodiana*, exposure, Cu, protein.

Abstrak. *Kurnia AI, Purwanto E, Mahajoeno E. 2010. Paparan logam berat tembaga (Cu) pada kerang air tawar (*Anodonta woodiana*) dan hubungannya dengan kandungan Cu dan protein dalam tubuh kerang. Nusantara Bioscience 2: 48-53.* Untuk mengetahui hubungan pemaparan logam Cu dalam air terhadap kerang air tawar maka dilakukan percobaan pemaparan dengan air yang mengandung Cu. Pengaruh yang diukur adalah kadar Cu dan kadar protein dalam tubuh kerang. Penelitian ini menggunakan kerang air tawar *Anodonta woodiana*. Kerang dipaparkan selama empat minggu dalam air dengan konsentrasi Cu 0.02 ppm, 0.04 ppm, 0.06 ppm dan kontrol 0.00 ppm. Kadar Cu dan kadar protein dalam tubuh kerang diperiksa setiap minggu. Analisis Cu dilakukan dengan metode AAS dan kadar protein menggunakan metode Kjeldahl. Analisis Cu menunjukkan peningkatan kadar Cu dalam tubuh kerang setelah pemaparan. Pola kenaikan kadar Cu tidak sama, dimana pola kenaikan terbesar terjadi setelah minggu keempat. Uji statistik menunjukkan tidak adanya pengaruh signifikan antara perlakuan dengan akumulasi Cu dalam tubuh kerang. Analisis protein menunjukkan kenaikan kadar protein setelah pemaparan minggu kedua dan menurun setelah minggu ketiga dan keempat. Pola perubahan kadar protein bervariasi antar berbagai perlakuan. Uji statistik menunjukkan tidak adanya pengaruh signifikan antara perlakuan dengan perubahan protein dalam tubuh kerang. Uji korelasi hubungan antara kadar Cu dalam tubuh kerang dengan kadar protein menunjukkan adanya korelasi positif antara keduanya dengan tingkat hubungan yang cukup baik (koefisien korelasi r = 0.836).

Kata kunci: *Anodonta woodiana*, pemaparan, Cu, protein.

INTRODUCTION

The idea of environmental pollution in Indonesian Act No. 23 of 1997 on Environmental Management is the inclusion of living things, matter, energy, and/or other components into the environment by human activities so that its quality decreases to a certain level that causes environment can not function as intended. One of the pollutants that are often found as contaminants in the aquatic environment that is detected in the organism body is heavy metals, copper (Wardhana 2001). Copper (Cu) is a metal that widely used in chemical industry, metallurgy, textiles and anti-rust paint (Effendi 2003). Like other heavy metals, Cu, is difficult to be soluted in the environment and it can enter the food chain through organisms that exist in the water. Aquatic organisms that are known to accumulate heavy metals are phytoplankton, zooplankton, class of fish, crustaceans (crustaceans) and mollusks or shellfish species (Wardhana 2001).

Freshwater mussels (*Anodonta woodiana*) are bivalves included in phylum mollusks that have two symmetrical shells. They live in the riverbed, channels, ponds, and lakes. They can have rough or smooth shell depending on the habitat where they live. Freshwater mussels are microscopic plant-eating animals and perform it by sucking water through a siphon and removing the particles. As a filter organism, freshwater mussels can serve to clean water and reduce algae, particles, toxic materials, and some diseases.

Freshwater mussel is an organism that can be used as biological indicators or bioindicator (EPA 2009). Bioindicators are organisms or biological responses that indicate the entry of certain substances in the environment. Species or species group for bioindicator are selected based on several factors which is; they are easily measured and give response observed in ecosystems, have specific response that is able to predict on how a species or ecosystem will respond to a certain pressure, measure the response with accuracy and precision that can be accepted based on knowledge about contaminants and characteristics (Mulgrew and Williams 2006).

Umar et al. (2001) which researched on the relationship between Cu in the aquatic environment with the content Cu in the body of sea shells *Marcia* sp. concluded that the higher the Cu in water and sediment, the higher the metal content of Cu accumulated by shellfish that live in the area. According to Sunarto (2007), there is a relationship between structure of microanatomy, the efficiency of gill function, morphology and condition of freshwater mussels *Anodonta woodiana* with Cd concentrations in water. So from that relationship and the condition of the shell morphology can be used as a bioindicator macroscopic beginning at *A. woodiana* due to exposure to heavy metals Cd. Stolyar et al. (2005) say that there are significant effects caused by heavy metals Cu to the protein of freshwater mussels. In the laboratory experiments on a freshwater mussel *Anodonta cygnea* shows that at the time of Cu exposure concentrations in water increased by 10 micrograms/liter, the protein content of Cu in Methallotionin will experience increased 100.7% compared to control groups of organisms (without any treatment of heavy metal exposure Cu).

To find out how the relationship of heavy metal Cu exposure concentration to *A. woodiana* a laboratory study is conductedf with observations of variable concentrations of Cu and protein content in the body of a freshwater bivalve *A. woodiana*. This study aims to: (i) know the relationship between exposure concentration of Cu in water with Cu concentration in the body of *A. woodiana*, (ii) to study the relationship between the concentration of Cu in water with the protein of *A. woodiana*; (iii) know the relationship between the concentration of Cu in the body *A. woodiana* with protein *A. woodiana*.

MATERIALS AND METHODS

Place and time of study

The study was conducted in three places. Acclimatization activities, maintenance, and treatment of *Anodonta woodiana* was conducted at the Laboratory of Environment STIKES Karya Husada Kediri, East Java. Analysis of heavy metal Cu content in the body *A. woodiana* was conducted in Chemical sub-Laboratory, Center Laboratory of MIPA for UNS Surakarta. Analysis of protein content in the body of *A. woodiana* was conducted at the Laboratory of Agriculture, Faculty of Agricultural Technology, Sebelas Maret University, Surakarta

Material

Materials research is a freshwater bivalve species *A. woodiana*. Samples were taken from Freshwater Fish Seed Board (BBI) Janti, Klaten, Central Java. Shells used are wit the following specifications size is 7-9 cm long with a total weight of 30-45 grams.

Research design

The design of the research done on this activity was completely randomized design (CRD), with 3 (three) concentrations of Cu exposure treatment plus 1 (one) controls. Type of concentration are:

(i) Group 1: 0.00 ppm Cu concentration (C0)
(ii) Group 2: Cu concentration of 0.02 ppm (C1)
(iii) Group 3: Cu concentration of 0.04 ppm (C2)
(iv) Group 4: Cu concentration of 0.06 ppm (C3)

Selection of exposure concentration of Cu is based on PAN Pesticide Database (2009) which states that the *lethal concentration* (LC50) for copper (CuSO (iv) toward fresh water mussel *A. woodiana* is of 0.1 ppm.

A four-time examination will be conducted to the four groups based on the length of exposure time, namely:

(i) Examination 1: end of week-1 (T1)
(ii) Examination 2: the end of week-2 (T2)
(iii) Examination 3: end of week 3 (T3)
(iv) Examination 4: end of week 4 (T4)

From this experiment 16 units of the experiment will be obtained (4 x 4 examination treatment group). In each sample, the variables observed are concentration of Cu in the shells *A. woodiana* and protein levels in the shells *A. woodiana.*

Procedures

Experimental research

The animals tested were taken from fish ponds in Janti, Klaten, Central Java then brought to the Laboratory of Chemical STIKES Husada Kediri, East Java. The first step is acclimatization. The shells were looked after in an aquarium filled with clean water as much as 15 liters. Each aquarium filled with 15 shells. The water in the aquarium was changed every 3 to 4 days. Mussels were fed every other day. The food provided is brand Takari manufactured by PT. Protein Prima Tbk. Feeding is done by making food that is still a solid into a liquid form and then dissolved into the water in the aquarium. During the acclimatization, if there are dead shells then they will be replaced with new ones. Acclimatization process was conducted for 2 weeks. And then, experiments on heavy metals Cu exposure was conducted. The standard solution containing heavy metals such as Cu in certain concentrations was put into the aquarium as planned. Each type of concentration was carried out in three aquariums.

Aquarium grouping is as follow: aquarium 1, 2, and 3 for the concentration of Cu 0 ppm (control), aquarium 4, 5, 6 are for 0.02 ppm Cu concentration, aquarium 7, 8, and 9 for 0.04 ppm and 10, 11, 12 for Cu concentrations of 0.06

ppm. Standard solution is inserted into the tank together with replacing the water. So during the exposure time, the shells will continuously be in the water containing copper in that amount. Every water replacement will be accompanied by water temperature and pH measure. Furthermore, mussels/shells are taken for analysis of Cu content and protein content at the end of week 1, the end of week 2, 3, and 4.

Analysis of Cu content

Shells samples were taken, opened, and its feces was dumped. And then the samples were weighed in analytical scale and the weights were recorded. Samples were placed in a glass beaker. Pour 5 mL of $HClO_4$ into the sample and let stand for 3-5 minutes. Add 50 mL of distilled water. The sample was heated to form a homogeneous solution and leaving a volume of 20 mL. Remove the sample from the heater. Then 5 mL HNO_3 was added. It was again heated for 10-15 minutes. Add 50 mL distilled water. Filter with filter paper and insert it into the sample bottle. And then the sample was injected into the Flammable Atomic Adsorption Spectrophotometer (FAAS). From the injection, the data on levels of Cu would be obtained (in units of ppm).

Analysis of protein content

Shells samples were taken, opened, and its feces was dumped. Samples were crushed with a blender until smooth, then given 50 mL of distilled water and stirred until homogeneous. As much as 10 mL of sample solution were taken and put into 100 mL of glass, diluted until it reached the mark. From this solution (point 1) it was taken as much as 10 mL and put into 500 mL Kjeldahl flask and add 10 mL of H_2SO_4. Added 5 g mixture of Na_2SO_4-HgO (20:1) for the catalyst. The solution was boiled until clear and continued boiling for 30 minutes more. Once cool, wash the Kjeldahl flask walls with water and simmer again for 30 minutes, then cooled. When it was cool it was then added with 140 mL distilled water and add 35 mL of NaOH-Na_2SO_3 and some granules of Zink. Then do the distillation. Distillate was gathered as much as 100 mL in Erlenmeyer tube containing 25 mL of saturated boric acid and a few drops of red indicator. Titrate the solution obtained with 0.02 HCl. Number of total N (% protein) was calculated with the formula as follows:

$$\text{The number N total} = \frac{\text{mL HCl x N HCl}}{\text{mL sample solution}} \text{ x } 14{,}008 \text{ x f}$$

f = dilution factor

Data analysis

Analysis of variance (ANOVA) is used to see if there is variation among the treatments. Rank data analysis with analysis variant will be conducted on data obtained from the examination of Cu content in the network and data examination of protein levels in the body *A. woodiana*. Correlation analysis is used to determine the relationship between variables observed in the study. Data to be tested with correlation analysis is the relationship between the concentration of Cu in water with high levels of Cu in the body *A. woodiana*, the relationship between the concentration of Cu in water with protein content in the body *A. woodiana*, the relationship between protein content with the content of Cu in the body *A. woodiana*.

RESULTS AND DISCUSSION

Relations between Cu concentration in water with the levels of Cu in the body

To describe the magnitude of changes in Cu levels in the body of the shells in each treatment the results are expressed as a histogram. Image histogram is shown in Figure 1 which shows the movement or changes in Cu levels in the body shells at each treatment concentration.

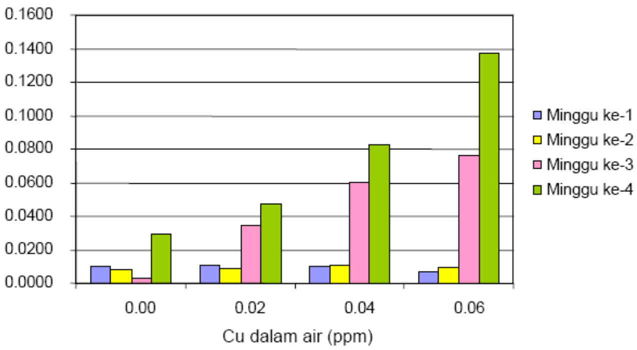

Figure 1. Cu levels in the body of shells *A. woodiana*

The ANOVA test on these calculations using degrees of freedom of 3 and with a confidence level of 95% (ρ = 0.05). From the table, ANOVA F test result greater than the F table. The results of this test gives the sense that there is no significant difference in Cu concentrations in water treated with Cu levels in the body shells, or the variation of treatment to be given in the form of concentration variation of Cu in the water did not give significant effect on levels of Cu in the body shells. Based on ANOVA statistical test the results stating that there is no significant difference in the concentration of Cu treatment. It means that the results of statistical tests can be analyzed from several points of the view.

According to Sărkăny-Kiss et al. (2000), the ability of mussels organisms to accumulate heavy metals in the body is influenced by two factors, namely *exterior complex* and *inner complex*. Exterior complex is the condition of water environment in which these organisms live. While the inner complex factors are matters related to the metabolic capabilities of organisms with the presence of heavy metal components in the body. Referring to the opinion, the phenomenon in this study can be viewed from two factors, namely the live shells is based on water conditions as the life media, and based on the characters of organisms *A. woodiana*.

In the factor of environmental, this experiment has been made causes the environment in which mussels live are relatively similar and homogeneous. All the variables and parameters associated with mussels habitat in this experiment have been controlled and made uniform. Only the concentration of Cu which is made varies according to the research design.

The first is about water. In this experiment, the water used to perform the experiment originated from the same water source. For each type of treatment, water used is handled in the same way and taken at the same time. Physical factors of the water as place for living such as temperature and pH was monitored in every water change. Water temperature during the experiment ranged in around 27^{o}C. The food given was also made similar for each treatment. In this experiment, mussels were fed every other day. Food provided is Takari fish feed manufactured by PT. Protein Prima Tbk. Each concentration treatment was the same food and made at the same time.

Based on existing levels of Cu in the body of samples mussels, it appears that among the individual samples there are significant differences in the ability to absorb copper, to process of metabolism and to accumulate copper particles that are soluble in water. Then this different ability results in different Cu accumulation in the body. This fact can be seen on the results of analysis of Cu for each sample (Figure 1). From this figure, it appears that the same type of treatment concentration and exposure at the same time still shows that there are a few samples that gave results that differ greatly. The difference of the Cu accumulation got here almost hit 100% difference. For example in the treatment concentration of 0.02 ppm Cu (week 2), Cu 0.04 (week-1 and 2) and Cu 0.06 at week 1. The same table also shows that from the same type of treatment and longer exposure time resulted in the lower accumulation of copper, while the other treatments showed an increase.

This is visible on the treatment concentration of 0.02 ppm, where in the first week its average Cu accumulation was 0.0107, but in the next week, the number decreased as much as 24.80% to 0.0086 ppm. Difference like that is what the statistics would cause the effect of exposure concentration of Cu in the water has no significant effect on Cu content in the body *A. woodiana*. A pattern of Cu accumulation in the mussel's body that vary at the same time shows that the system of mussels biology and metabolism is not a simple system which has a linear mechanism, where the same input (treatment) would results that were very different.

According to Krolak and Zdanowski (2001) the fact that the Cu content varying in individual of *A. woodiana* living with the same treatment is the effect of a selective retrieval capabilities of various components in the surrounding environment and is influenced also by the ability of different parts of each organ in the body of the mussels to accumulate Cu.

From these quotations steps of analysis can be taken that the Cu content accumulated in *A. woodiana* after exposure to the metal Cu is influenced by two things. These two things are how the Cu ions get inside of the organism and how the Cu ions are accumulated in the organism. It can be said that the difference in levels of Cu accumulated in this experiment was caused by the differences in both processes.

The process of inclusion of Cu ions into the mussel's body.

In Soto et al. (2008) it is explained that metal enters the oysters in two ways. First is through the gills and second through the organs of the body which is in direct contact with the water containing the heavy metals. From these quotations, we can say that the quantity of Cu goes into the shells is influenced by two things. The first is related to the gills and the second is related to the contact or exposure of organs with heavy metals.

If Cu enter through the gills: in bivalves, including Anodonta wodiana, metal ions in water will get into the gills by diffusion, which is a more passive process (Soto et al. 2008). Because this process is passive, the decisive part of the diffusion process is the condition of the gills, i.e., the width of the filament surface. If the greater the surface of gill filaments, then the diffusion will occur the more and more, thus more Cu ions enter the body of the mussels.

Besides the diffusion process, the process of Cu enters the gill also involves active transport mechanism, i.e., when the gills must work against the pressure difference between the water pressure and the pressure in the gills. Gill's ability to perform *active transport* depends on the availability of ATP (Soto et al. 2008). From the description of this process shows that the extent of Cu entering through an active transport process depends on each individual. Where the influential part is the availability of energy in the form of ATP.

Cu influx through the organ of the mussels which is in direct contact with the water. In *A. woodiana*, organ which is in direct contact with water is the organs which anatomically Fox calls (2005) the *external anatomy*, that is the shell, muscle cells *(mantle)*, gill, the body (*visceral mass*), foot, and *labial palps*. In the closed shell condition, the organs which will be in contact with water are the two pieces of shells, while the organs that are inside it are not in contact with water. In the condition where the shell is open, all the organs of *external anatomy* will be in contact with water.

At the time of contact with the media/water, the Cu ions dissolved in water will penetrate the surface of the organ and enter into the cell. The process when Cu ions enter into the cell occurs in two ways, namely by passive diffusion and active transport. In the process of passive diffusion, the parts that play important role are the area in contact with water. While on active transport processes the most influential thing is the availability of energy (Soto et al. 2008).

From these conditions, the process of Cu entering through the contact is determined by the surface area of the water contacts. The greater the surface area that contacts the more and more the Cu that can enter the cell. Similarly, the greater the available energy of ATP, the more active transport process and Cu will be more included in the cell.

The process of Cu ion accumulation in the body of the mussels

After the copper enters the body, on a scale of cellular the metals will experience stabilization process, from the original shape that is still in the form of free ions then form a ligand binding with other components. Cu bond stabilization process in the body bivalves takes place in two processes. The first is in the process of synthesizing metal-binding proteins, where metal ions play role as promoter and will be bound in a protein that is formed. The second process is the formation of granulation of mineral grains or *mineralized granules*, in which metal ions would be bound in mineral granules that are not dissolved (Soto et al. 2008). According to Stanley (2003) the process of stabilization and detoxification of heavy metals in the body are made in three types of processes, namely: (i) through the formation of soluble compounds that bind metals, in this case it is methallotionin protein synthesis, (ii) through the storage process *(compartmentalization)* of metal in one of the cell organelles cell which is in lysosomes, (iii) through the formation of the precipitate that was dissolved in the form of granulation of mineral grains that can bind metals.

When it is known that the metal accumulation process is done in two or three streets, as mentioned above, when the results of this study found no significant relationship between metal concentration exposure to metal accumulation in *A. woodiana*, meaning three Cu accumulation process occurring in the body are varied and not uniform. This variation that ultimately gives the resultant in the form of accumulated Cu levels varying between individual samples of shellfish. The existence of these differences were in line with Viarengo et al. (1987) who said that the detoxification process that involves the stabilization and the storage of metals in the body of bivalves have a level of effectiveness that varies among species, in the same species between different individuals and on the same individual between different organs. These differences, according to Soto et al. (2008) defined by two biotic factors, namely the age and the weight of individual. Age influences the level of sensitivity of shellfish organisms to absorb metal ions. The young individuals are more sensitive and able to absorb heavy metals more than the older individual. While the weight ratio between weight and volume of organs which can be exposed by metal.

Relations between Cu concentration in water with levels of protein in the body of the mussels

Results of analysis of protein levels in the body are the result of the examination of laboratory analysis of total protein content in samples of shellfish *A. woodiana*; presented in Figure 2, which shows the changing levels of protein in the body shells in each - each concentration treatment.

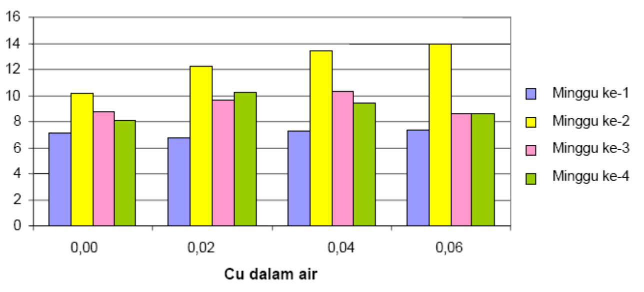

Figure 2. Protein levels in the body of *A. woodiana*.

From the table ANOVA, F test result is greater than the F table. The results of this test give the sense that there is no significant difference in differences of treatment Cu concentration in water with levels of protein in the body of the mussels. The variation of treatment provided in the form of various concentration of Cu in the water does not have a significant impact on levels of Cu in the body of the mussels.

In the study of the relationship between the effects of heavy metals with the change in levels of protein in organisms, the review is on protein Methallotionin. According to Soto et al. (2008) Methallotionin protein (MT protein) is a Cytosol protein with low molecular weight, soluble, resistant to high temperatures *(thermophilic proteins)*, rich in sulfur elements (more than 30%) and has a strong affinity with metal ties. In aquatic organisms, MT proteins responsible for maintaining the metal concentration remain at low levels. Protein synthesis was induced by the presence of metal in the cell. MT protein specifically binds with metal Cd, Cu, Hg, and Zn ions. Increase in levels of MT proteins associated with an increase in the capacity of cells to bind heavy metal ions which increases the protection against toxicity of heavy metals.

In this study, based on statistical tests it is concluded that there is no significant impact from the variation of Cu in water treatment to changes in protein levels in mussels. In other words that the difference of treatment and duration gave no significant impact on the changing levels of protein in the body of the mussels.

The result of these statistical tests can actually be read visually from the pattern of change protein content. In Figure 2. above it appears that there is an increase of protein levels in the second week of exposure. After that in week three, the protein levels decreased and in the next week, there was also a decline, except in the 0.04 ppm treatment the protein content increased in the fourth week. The existence of patterns of change that are not regular showed a wide variation that occurred in this experiment. The results of this study more or less are in line with Stolyar et al. (2005) on Cu exposure toward *Anodonta cygnea*. In the study it is illustrated that at low concentrations of Cu exposure, increase in levels of protein will be the same as the control organisms or, no change at all. While the highest concentration of Cu exposure (0.2 ppm) did not provide a significant impact on changes in protein levels in the body of the mussels that were tested.

The reasons of why there is no relationship between levels of Cu in the water with the change of protein in the body of the mussels can be seen in two views. The first refers to the opinions of Stanley (2003). In this study, it is explained that the heavy metals are stored in the body of freshwater mussels in three forms, namely as a metal-bound in the precipitate minerals, metals stored in cell organelles as lysosomes and metal that is bound by the metal-binding-protein binds, or Metallothionein. Soto et al. (2008) add that in the body of freshwater mussels there is also the ability to excrete heavy metals that enter directly, besides the three processes mentioned above.

From the statement above, the presence of protein synthesis formed in response to the entry of metal into the cell is one of four other responsive processes. In other words, the existing copper metal in water will not fully trigger a protein synthesis in cells. That is because there are other mechanisms of storage in the lysosomes, the process of granulation with a mineral and excretion of Cu ions directly. In addition, up to now, it has not been known for certain about which one is the most dominant process in response to the entry of copper ions into the body of the mussels.

Relations between Cu concentration in the body with levels of protein in the body of the mussels

The relationship between Cu levels in shellfish with high levels of protein in the shells is presented in Figure 3 below:

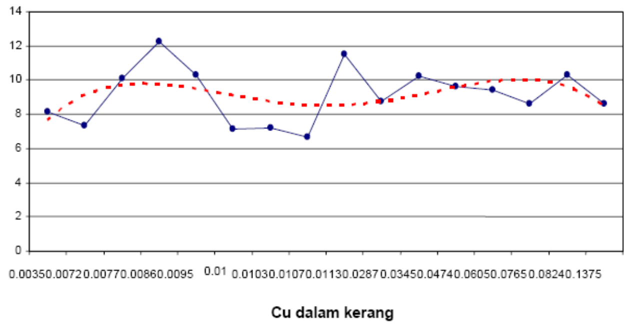

Figure 3. The relationship between Cu content with protein content in the body of A. woodiana

Figure 3 shows that there are variations in the pattern of relationship between Cu with proteins in the body shells. At 0.0035 ppm Cu values up to 0.0107 ppm, protein content has a pattern of relationships that fluctuate up and down. Meanwhile, in the range, 0.0287 ppm Cu values up to 0.1375 ppm, which was conceived by the protein content of oysters tend to have patterns of increase with increasing Cu content. To determine the relationship between Cu content with protein content in the body shell, then the data were analyzed by correlation method of Pearson Product Moment (Nazir 2005).

The computation with the working table obtained that the correlation coefficient amounted to 0.848. This value illustrates the positive correlation between two variables measured, namely Cu levels in the body shells with protein levels in the body shells. Where the degree of relationship is quite good. In other words, the change of Cu content in the body shells have a fairly strong correlation with changes in protein levels in the body shells.

These results are in line with the opinion delivered by Couillard et al. (1993) who argued that in studies with bivalve species, *Anodonta grandis* subjects showed a strong correlation between elevated levels of Cu in the body with protein content in the cell body of organisms such shells. These results are in line with research conducted by Stolyar et al. (2005) which illustrates that copper metal ions in water will increase the metal content in the cells of the body. And it will lead to increased levels of MT proteins in animals *Anodonta cygnea*. Soto et al. (2008) explain the elevated levels of Cu linkages with elevated levels of this protein. Increased levels of this protein are a response to elevated levels of Cu, where MT protein will bind Cu ions to prevent the toxicity of these metals.

CONCLUSION

Provision of Cu concentration in water did not affect significantly to the amount of Cu content accumulated in the body of *A. woodiana*. Provision of Cu concentration in water did not give significant effect on changes in body protein content of *A. woodiana*. Cu levels in the body have a positive correlation with levels of protein in the body of *A. woodiana*.

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