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Microanatomy alteration of gills and kidneys in freshwater mussel (*Anodonta woodiana*) due to cadmium exposure

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Abstract. *Fitriawan F, Sutarno, Sunarto. 2011. Microanatomy alteration of gills and kidneys in freshwater mussel (*Anodonta woodiana*) due to cadmium exposure. Nusantara Bioscience 3: 28-35.* The purpose of this study was to determine the level of Cd accumulation in the gills and kidneys, to know the changes in microanatomic structure of *A. woodiana* after the various treatments of heavy metals. Completely randomized design pattern of 5 x 3 as used in this laboratory experiment. The amount of exposure of heavy metals Cd was (0 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm), while the variation of length of exposure time to Cd were (7 days, 14 days, and 30 days). The parameters of Cd accumulation in the gills and kidney was analyzed by using AAS method, while abnormalities of gills and kidney were detected by microanatomy structure. Data collected were then analyzed using the analysis of variance (ANOVA) and continued with further test the DMRT. The results indicated that there is a significant effect in 475.3 > 0.000 and 60150.3 >0.000 with 5% significance level ($P<0.05$) of Cd treatment on gill and kidney microanatomy of *A. woodiana*. The changes in microanatomy structure of those organs are including edema, hyperplasia, fusion of lamella, necrosis, and atrophy.

Key words: *Anodonta woodiana*, cadmium, gills, kidneys

Abstrak. *Fitriawan F, Sutarno, Sunarto. 2011. Perubahan mikroanatomi pada insang dan ginjal kerang air tawar (*Anodonta woodiana*) terhadap paparan kadmium. Nusantara Bioscience 3: 28-35.* Tujuan penelitian ini untuk mengetahui tingkat akumulasi, perubahan struktur mikroanatomi setelah perlakuan logam berat Cd pada insang dan ginjal *A. woodiana*. Jenis penelitian yang digunakan yaitu eksperimental laboratorium dengan rancangan acak lengkap (5 x 3) berupa besarnya paparan Cd (0 ppm, 0,5 ppm, 1 ppm, 5 ppm, 10 ppm) dan waktu pemaparan Cd (setelah 7 hari, 14 hari, dan 30 hari). Parameter pengujian mencakup uji akumulasi Cd pada insang dan ginjal dengan metode AAS, dan abnormalitas insang dan ginjal akibat akumulasi Cd dengan metode preparasi. Analisis akumulasi Cd pada insang dan ginjal menggunakan analisis varian (ANAVA) dan dilanjutkan dengan uji lanjut jarak berganda Duncan (DMRT). Hasil penelitian menunjukkan pengaruh pemberian beberapa perlakuan Cd terhadap kontrol insang dan ginjal *A. woodiana* signifikan sebesar 475,3 > 0,000 dan 60150,3 > 0,000 dengan taraf signifikansi rata-rata 5% ($P<0,05$) yang ditandai dengan perubahan struktur mikroanatomi pada insang berupa edema, hiperplasia, fusi lamella, nekrosis hingga atropi. Sedangkan pada ginjal berupa edema, hiperplasia dan nekrosis pada tubulus, glomerulus, dan mineralisasi pada sel darah hingga mengalami pendarahan.

Kata kunci: *Anodonta woodiana*, kadmium, insang, ginjal

INTRODUCTION

Cadmium (Cd) is one type of heavy metals that are useful in several industries. For example in the textile batteries industry and electroplating, as coloring matters in ink. Cd also exists naturally in foods even if only in small amounts absorbed by the intestine (5-8%) (Palar 1994). But on the other hand, heavy metal can cause problems; problems can occur more severe if waste management is not done properly, so that it will have an impact on the environment as a micro-pollutants (Soegianto et al. 2004). Incoming cadmium in fresh water will be joined with a metal ion cofactor so that the shape of Cd^{2+} causes the toxicity of the water. Cd^{2+} 's toxicity levels in water depend on salinity. The toxicity of Cd^{2+} in the water will rise if the salinity is low.

To determine the level of pollution in a region we can use a particular bioindicator organism typical of one that can be used, namely *A. woodiana*. The advantages of this animal are that they settle in one place, and have a slow movement, so that if an environment is exposed to heavy metal waste Cd then indirectly affects the lives of these biotas. Cd accumulation in an organism other than cause exposure to the organ, it will also cause interference on enzyme activity. The nature of the toxic metal is due to its very effective in binding itself with the group of sulphurhydril (SH) in the enzyme system of cells that form bonds and metalloprotein metalloenzyme so that enzyme activity for cell life processes cannot take place (Connell and Miller 1995).

Gills and kidneys are vital organs. Gills play a role in the process of respiration, acid-base balance, ionic and osmotic regulation because of the branchial epithelium tissue which became the meeting place of active transport between organisms and the environment (Soegianto et al. 2004). Renal function begins in the glomerular ultrafilter that is formed from the plasma. Ultrafilter will enter the Bowman's capsule and into the lumen of the tubule. Filtering through the various segments of the tubules causes changes in the volume and composition of fluid filtration as a result of the process of reabsorption and secretion along the tubules (Tresnati et al. 2007). Glomerulus is composed of blood capillaries to function as a selective filter from the blood mainly in the normal blood screening (Takashima and Hibiya 1995). Following through on glomerular filtration and being re-absorbed in the tubular, it produces urine as a result of secretion in normal circumstances (Tresnati et al. 2007).

The purpose of this study is: (i) to know the content of Cd accumulation, (ii) changes in the microanatomy structure, and (iii) in gill and kidney of *A. woodiana* after treatment.

MATERIALS AND METHODS

Time and place

The research of the Cd treatment on *A. woodiana* conducted at the Laboratory of Pharmacy and Food Academy Analyst Sunan Giri, Roxburgh. Analysis of heavy metal content by AAS method was carried out in sub lab Chemistry Laboratory of Mathematics and Science Center UNS Surakarta, while the preparation for the analysis was conducted in the laboratory animal anatomy Faculty of Veterinary Medicine, Gadjah Mada University in Yogyakarta. The experiment was conducted in October-November 2009.

Materials

Freshwater mussels (*A. woodiana*) were obtained from farms in the fishing village of the tourist site of Janti, Polanharjo Subdistrict, Klaten District, Central Java.

Procedures

Freshwater bivalve *A. woodiana* was selected based on the maximum growth and uniform size. The shellfish were acclimatized for 15 days, after which it was examined by using the compound of Cd for 30 days with repeated 3 times at day 7, 14 and 30. Physical-chemical parameters measured include pH, DO and water temperature. Cd content of the examination conducted on the gill and kidney *A. woodiana* with AAS method. Preparation of gill and kidney preparations performed with hematoxylin-eosin (HE) method with treatment stages, i.e. trimming, dehydration, embedding, cutting, staining, mounting and reading the results.

Data analysis

Environmental chemistry parameters (pH, DO, temperature) were described with a descriptive method. Effects of Cd exposure on the gill and kidney *A. woodiana* were analyzed by ANOVA one-way significance level of 5% ($P > 0.05$), followed by a further test of significant difference or Duncan's multiple range test (DMRT). Abnormality in the microanatomy structure of gills and kidneys of *A. woodiana* was directly observed and described with a descriptive method.

RESULTS AND DISCUSSION

Water environmental parameters

Examination of physical and chemical parameters of water quality used in this study include the degree of acidity (pH), dissolved oxygen (DO), and water temperature.

Degree of acidity (pH)

The degree of acidity or pH is a value that shows the activity of hydrogen ions in water. The pH of water reflects the balance between acid and base in these waters. The pH range 1-14, pH 7 is the boundary halfway between the acid and alkaline (neutral). The higher the pH of the water, the greater the base nature will be, and the lower the pH, the more acidic the water. PH value is influenced by several parameters, including biological activity, temperature, oxygen content, and ions. From the biological activity, CO_2 gas is generated as a result of respiration. This gas will form a buffer or buffer ions to maintain the pH range in the waters in order to remain stable.

In this study, the pH is very important as water quality parameters, for controlling the type and rate of speed of reaction some materials in the water. In addition, *A. woodiana* lives at a certain pH interval, so that by knowing the value of pH, it can be known whether or not the water supports their lives. Based on Figure 1A, it is known that the higher Cd concentration, the higher the value of the range of pH waters. On day 7, pH values ranged from 7.34 to 8.44, on day 14 ranged from 7.37 to 8.40, and the day-to-30 range from 7.31 to 8.68. According to Erland (2007), pH to function as an index of environmental conditions and limiting factors, where each organism has a different tolerance to pH maximum, minimum and optimal.

According to Erland (2007) pH value of water has a special characteristic, the hydrogen ion concentration be measured by the balance between acids and bases. Acid-free mineral acid and carbonic acid will lower pH value (acid), while the carbonate (CO_3), hydroxide (OH-) and bicarbonate to raise pH (alkaline). Rochyatun et al. (2006) states, that at a relatively high metal content will be alkaline pH values (pH 7.40 to 8.59), where the metal is difficult to dissolve and settle to the bottom of the water. When in the treatment, pH values from 0.5 to 10 ppm, in the study it ranged from 7.92 to 8.68, indicating water has been polluted quite heavily, with the level of alkalinity in excess of tolerance. According to Connell and Miller (1995) increase in pH in the waters will be followed by decreasing the solubility of heavy metals that tend to settle. Deposition can occur in sediments and food; the food will enter and accumulate in the body of *A. woodiana*. Given the Cd is a non-essential metal that cannot be degraded so that it will cause interference with the organs, such as the gill and kidney.

Water solubility of oxygen (DO)

Oxygen is one of the gases dissolved in natural waters with varying levels are influenced by temperature, salinity, water turbulence, and atmospheric pressure. Besides necessary for the survival of aquatic organisms, oxygen is also needed in the process of decomposition of organic compounds. Sources of dissolved oxygen are mainly derived from the diffusion of oxygen from the atmosphere. This diffusion occurs directly on stagnant conditions (silent), or because of agitation (water mass unrest) caused by waves or wind.

Figure 1B shows that the higher concentration of Cd treatment, then progressively decreasing levels of dissolved oxygen (DO) in water. Ardi (2002) classified water quality based on the DO into four types namely; not contaminated (> 6.5 mg/L), lightly polluted (4.5 to 6.5 mg/L), being contaminated (2.0 to 4, 4 mg/L) and heavily polluted (<2.0 mg/L). In this study, the DO in the first test of 10.10 ppm decreased to 3.19 ppm, in the second test from 10.11 ppm to 3.25 ppm, and the third repeat of 10.26 ppm to 3.76 ppm. From the above results, the pollution can still be considered moderate.

Decreased levels of oxygen in the water are inversely proportional to the high Cd treatment in these waters. Cadmium is an inorganic contaminants/minerals that can accumulate in water or food. In general, the Cd that entered the waters will be Cd^{2+} which causes the toxicity waters, and the presence of sediment in the diet will be very easy to be consumed by aquatic biota, including *A. woodiana*. Solubility of oxygen is essential for the sustainability of aquatic life, oxygen is used as a tool of biota metabolism so it can carry out their duties as decomposer and degrading organic materials in order to more easily broken down by bacteria (Warlina 2004; Ardi 2002). If an aquatic polluted by a heavy metal inorganic, then *A. woodiana* not able to decompose organic materials, so that the decomposition process is highly dependent aerobic bacteria that need oxygen is very high, and can cause a deficit of oxygen in these waters.

According to Destiany (2007) with increasing concentrations of heavy metals, the dissolved oxygen content will decrease, and the CO_2 will rose, due to low oxygen levels that require aquatic biota such as *A. woodiana* to pump water through their gills, thereby increasing the rate of respiration and dissolved CO_2 increases, so the toxins more and more absorbed in the body through the gills. The higher the level of aquatic toxicity, the higher the rate of breathing will be (Budiono 2003).

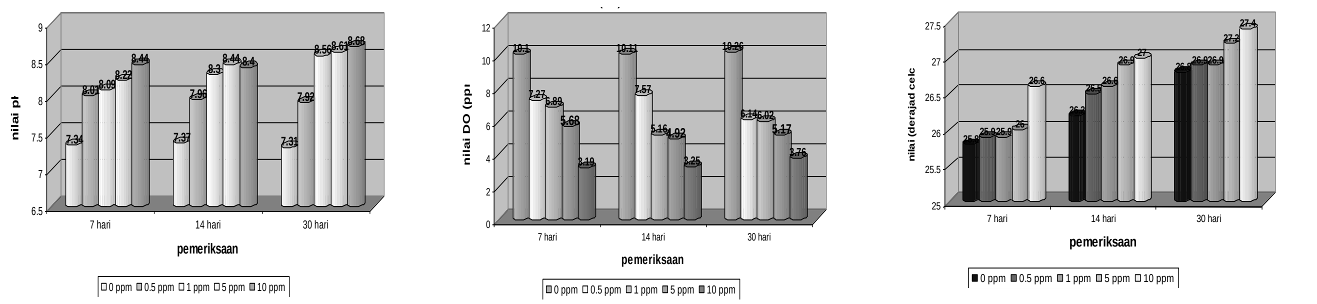
Dissolved oxygen is essential for respiratory zoobenthos and other aquatic organisms (Odum 1993). In addition, the solubility of oxygen is also affected by temperature, at high temperature and low oxygen solubility at low temperatures the high oxygen solubility. Each species of aquatic biota have a range of different tolerances to the concentration of dissolved oxygen in the water. Species with wide tolerance range and wide distribution of species with narrow tolerance range only live in certain places. Budiono (2003) stated that the excessive presence of heavy metals in the waters will affect the respiratory system of aquatic organisms, causing low dissolved oxygen levels, which disturb the life of aquatic organisms.

Temperature

Each of aquatic organisms has different tolerance limits to changes in water temperature to the life and growth of aquatic organisms. Therefore, the temperature is one factor that physically is very important for aquatic organisms or aquatic life. In general, the temperature directly affects the aquatic biota of enzymatic reactions in the organism and does not directly influence the structure of organs and the spread of aquatic animals (Nontji 1984).

From Figure 1C, it is known that the temperature of different water looks increasingly high Cd treatment at each treatment, which ranged from 25.8 to 26.6°C on the first test, and the second test ranged from 26.2 to 27°C, the third test ranged from 26.8 to 27.4°C. This is influenced by metal accumulation in each treatment with the higher concentration, thus causing the water temperature value is also higher. It is inversely proportional to the solubility of oxygen in water, i.e. at high-temperature low oxygen solubility, and low solubility of oxygen at high temperature (Odum 1993).

The Relationship between the temperature rise of heavy metal accumulation in the water is strong. Cd is an inorganic non-essential metal that cannot be in the degradation of benthos organisms and microorganism. The presence of metal causes the metabolic rate of aquatic biota



A B C

Figure 1. The physical-chemical parameters condition of waters in the experimental location after administration of Cd. A. the degree of acidity (pH), B. DO, C. temperature.

increased in order to defend themselves, so that automatically the oxygen demand is very much while on the other hand, given the concentration of heavy metals higher, thus increasing the concentration of heavy metals that enter the more carbon dioxide (CO_2) are released that cause the oxygen content dwindling waters so that the rising water temperature.

According to Connell and Miller (1995), the role of water temperature is very important to help the body's metabolism of aquatic animals. The increase in water temperature can cause the immune system of aquatic biota to decrease. So if a toxic Cd^{2+} enters the body of *A. woodiana* the biota will be very difficult to retain yourself from the poison.

Accumulation of Cd in the gills of A. woodiana

The result of the content of Cd in the gill and kidney *A. woodiana* with AAS method are shown in Table 1. From the results, it is known that the increasing Cd treatment, the increase of Cd accumulated in the gill of *A. woodiana*. In the control (0 ppm), Cd accumulation in the gills of *A. woodiana* was 0.12 ppm, the accumulation in control is still below the maximum tolerance limit Cd accumulation in organs, as specified by the FAO (1972) and MOH (1989), namely a maximum accumulation of Cd in organs of 1 ppm. It is also in accordance with preliminary studies that have been made to the content of Cd in water samples, with the result that the content of Cd in Janti aquaculture that was still in the normal state that was 0.0028 ppm (IGR No. 82/2001; EPA 1986).

After the examination after 7 days the average values obtained Cd accumulation in gill *A. woodiana* in the treatment of 0.5 ppm was 0.58 ppm, treatment of 1 ppm was 0.87 ppm, 5 ppm was 1.00 ppm and 10 ppm was 2.15 ppm. Meanwhile, after the examination on day 14, obtained an average value of accumulated Cd at 0.5 ppm treatment was 0.78, treatment of 1 ppm 0.93 ppm, 5 ppm treatment at 1.24 ppm, and on treatment 10 ppm at 2.34 ppm. After the examination on day-30, it was obtained an average value of Cd accumulation in the treatment of 0.5 ppm 1.43 ppm, 1 ppm treatment at 1.01, 5 ppm treatment at 2.58, and the treatment of 10 ppm 3.49 ppm.

Darmono (1995) states that the relationship between the amount of metal absorption and metal content in water is usually in proportion, the increase in metal content in the network in accordance with the increase of metal content in water. According to Sunarto (2007), Cd will also experience the process of biotransformation and bioaccumulation in aquatic biota. Cadmium enters the body along the water or food consumed, but Cd has contaminated water or food. The amount of metal that accumulates in the gills will continue to increase, even very likely continue to enter through the accumulation of Cd digestive tract to the kidney; in addition to increasing levels of pollutants in the presence of Cd may also biomagnification process in the water body. If the amount of Cd that enters the body and has exceeded the threshold value, it will experience death and even extinction.

Cd treatment against gill *A. woodiana* with a concentration of 0 ppm, 0.5 ppm, 1 ppm, 5 ppm, and 10 ppm for 7 days, 14 days and 30 days to yield significant results ($P < 0.05$). This is in accordance with the opinion Darmono (1995) which states, that the relationship between the amount of metal absorption and metal content in water is usually in proportion, the increase in metal content in the network in accordance with the increase of metal content in water.

ANOVA test and Duncan's range test showed no association of Cd accumulation in gill of *A. woodiana* with the concentration of Cd (Table 1). The higher the concentration of Cd is given, the higher the exposure to Cd on the gills of *A. woodiana* will be. The most obvious difference is indicated by the treatment concentration of 10 ppm, which is the highest concentration, thus giving an average value of accumulated most Cd concentrations higher than below it. As for the concentration of 0.5 ppm and 1 ppm, obtained test results are less tangible difference, possibly because the treatment they are not too much difference compared with other treatments, so that the results of cadmium exposure on *A. woodiana* not too apparent.

Table 1. Treatment test results in the accumulated Cd concentration of Cd in gill and kidney *A. woodiana*.

Treatment of Cd concentration (ppm)	Average Cd accumulation in gill (ppm)	Average Cd accumulation in kidneys (ppm)
0	0.12 a	0.018933 a
0.5	0.93 b	0.045200 b
1	0.94 b	0.042956 b
5	1.61 c	0.082844 c
10	2.66 d	0.660111 d

Note: Value having the same letter notation means that no effect significantly different

ANOVA test and Duncan's range test that also shows a relationship between the length of the treatments with the level of Cd accumulation in the gill of *A. woodiana* (Table 2). The longer treatment time means the accumulation of Cd in the gills. In Table 2, It can be seen that a 30-day treatment gave the highest average of the Cd accumulation on the gills of *A. woodiana*. Then further test the distance from Duncan to get the illustration relations and exposure levels of Cd accumulation in the gill of *A. woodiana* to the long treatment, where the old high Cd treatment is given, the higher accumulation of Cd in the gills of *A. woodiana*.

Table 2. Old test results in the treatment of Cd accumulation in gill and kidney *A. woodiana*

Length of treatment (days)	Average Cd accumulation in gill (ppm)	Average Cd accumulation in the kidney (ppm)
7	0.940313 a	0.048420 a
14	1.081420 a	0.063740 a
30	1.728753 b	0.397867 b

Note: Value having the same letter notation means that no effect significantly different

Analysis of treatment outcome of the renal Cd of *A. woodiana*

Test results on the Cd content of the kidney *A. woodiana* after treatment is shown in Table 1. The average value of the kidney of *A. woodiana* in the control group amounted to 0.019 ppm (0.018933 ppm), the value accumulated in the control was still below the maximum tolerance limit Cd accumulation in organs, as specified by the FAO (1972) and MOH (1989) that is equal to 1 ppm. It is also in accordance with preliminary studies that have been made to the content of Cd in the water, that the content of Cd in the water of Janti aquaculture is still in the normal state is 0.0028 ppm (IGR No. 82/2001; EPA 1986).

Later in the treatment of 0.5 ppm Cd in the kidneys after examination, AAS average accumulation after 7 days was at 0.020 ppm, after 14 days was at 0.029 ppm, after 30 days was at 0.086 ppm. Later in the treatment of 1 ppm after 7 days accumulation of 0.030 ppm, 0.031 ppm after 14 days, and after 30 days at 0.066 ppm. Later in the treatment of 5 ppm Cd accumulation after 7 days at 0.057 ppm, after 14 days at 0.085 ppm, and after 30 days at 0.107 ppm. And in the treatment of 10 ppm Cd accumulation in the kidney after 7 days at 0.116 ppm, after 14 days at 0.150 ppm and after 30 days showed the exposure of 1.717 ppm. From the above data, it is known that the higher the concentration of Cd treatment *A. woodiana*, the higher the value of exposure to cadmium in the kidneys of *A. woodiana*. This is similar to what has been mentioned by Sunarto (2007) that Cd will also experience the process of biotransformation and bioaccumulation in aquatic biota. Cadmium enters the body along the water or food consumed, where Cd has contaminated water or food. The amount of metal that accumulates in the gills will continue to increase, even very likely continue to enter through the accumulation of Cd digestive tract to the kidney.

Based on ANOVA statistical test, it is known that Cd treatment on the kidney of *A. woodiana* with a concentration of 0 ppm, 0.5 ppm, 1 ppm, 5 ppm, and 10 ppm for 7 days, 14 days and 30 days, yield significant results ($P < 0.05$). This is in line with the significance indicated on the gills of *A. woodiana*. Furthermore, Duncan range test indicated that the higher the concentration of Cd treatment, the higher the accumulation of Cd in the kidneys of *A. woodiana*. Treatment of Cd concentration of 10 ppm gave the highest cumulative impact with the most obvious significance level, of the treatment concentration underneath. Later the treatment of 0.5 ppm and 1 ppm did not show significant differences, it might be that the range of Cd concentrations that had been given is not far adrift, so that the value of exposure and accumulation of Cd in the kidneys was directly proportional to the treatment given. Cd concentration of 10 ppm gave the most obvious difference.

To know the effect of long exposure to the treatment of kidney due to accumulation of Cd in *A. woodiana*, it was also performed ANOVA test followed by a significantly different test (Duncan), where the longer treatment time means the higher the impact on exposure accumulation of Cd in the kidneys of *A. woodiana*, where this long a period of 30 days of treatment gave the greatest average rating of accumulation.

According Destiany (2007) said that the process of accumulation of chemicals in living things is described as follows: foods that accumulate heavy metals such as cadmium, will be eaten by aquatic biota, including the types of bivalves and will enter into the digestive tract. From within the digestive (gastrointestinal) through the walls will go to the circulatory fluid, then after the circulatory fluid would most foodstuffs in metabolism and partly met with several networks, so it will be in storage in fat tissue. Then chemicals such as cadmium in the fluid circulatory oxidized to Cd^{2+} that cause toxicity and will accumulate in the liver, because the nature of the Cd is a material non essential, its presence in the liver cannot be inactivated by the enzyme, so it continues to settle the kidneys and create sediment there.

Microanatomical changes in the gill of *A. woodiana* after exposure to Cd

Accumulation of Cd has caused a variety of physiological damage to the organs of *A. woodiana*, because the nature of the toxicity of Cd accumulated in the body has exceeded the maximum threshold of 1 ppm (FAO 1972), where the metal LC50 at 3 ppm occurs 48-72 hours after treatment (Kraak et al. 1992). In this experiment, some damaged organ/tissue shrinkage as siphon, foot, gill, and kidney. According to Palar (1994), Cd can damage aquatic biota in the physiological system urinary system, gill, kidney, and blood circulation. Damage caused by Cd contacts continuously through the cell membrane results in the degeneration of the membrane. If Cd enters through the gill, the gill will cause the deficiency so that the body's metabolic function gets disrupted.

Result analysis of changes in cellular of the microanatomical structure of the gill and kidney of *A. woodiana* is shown in Table 3. From the data results of the transverse slice preparations of the gill of *A. woodiana* after accumulation of Cd, it is known that the symptoms of cell damage on the gill was known at a concentration of 0.5 ppm with marked with edema in the lamella brachialis, so that on day 14 and day 30 the hyperplasia was more visible impact. The worst damage at the cellular level of gills occurred on the treatment of 10 ppm, where the gills showed symptoms of edema that was accompanied by hyperplasia, and eventually the entire network of experienced fusion up to each lamella having atrophy (Figure 2).

In the normal gill at the concentrations of 0 ppm, with a concentration of 0.1004 to 0.1321 ppm Cd accumulation (Figure 2A), it can be seen all the parts of cells from epithelial cells, basement membrane, Lacuna, the blood cells until the cell pillar that were still in normal circumstances. Each of biota samples has carried the accumulation of these metals from the sampling site that is in the area of aquaculture of Janti. So to make a sample without the accumulated metal is extremely difficult. In addition, according to Rahman (2006) in general, heavy metal content in a body of water is different form the one with heavy metals that have been dissolved in the aquatic sediments especially heavy metals in the organ. A heavy metal when the waters would go down and settles to form sedimentation, it will cause the organisms that eat at the bottom waters, such as *A. woodiana* (bivalves) will have a great opportunity for exposure to heavy metals that have been bound and form sediment.

Table 3. Changes in cellular structure microanatomy A. Gill woodiana after exposure to heavy metals cadmium with HE staining preparation.

Concentration (ppm)	Time of surgery (days)	Edema	Hyperplasia	Fusion of lamella	Necrosis	Atrophy
0	7	-	-	-	-	-
	14	-	-	-	-	-
	30	-	-	-	-	-
0,5	7	+	-	-	-	-
	14	++	+	-	-	-
	30	+++	++	-	-	-
1	7	++	+	-	-	-
	14	++	++	++	-	-
	30	++++	+++	+++	-	-
5	7	++++	+++	+	-	-
	14	++++	++++	+++	-	-
(dead)	30	++++	++++	++++	+++	-
10	7	++++	+++	+	-	-
	14	++++	++++	++++	++++	++
(mati)	30	++++	++++	++++	++++	+++

Note: -: no change in the microanatomical structure (0%); +: there was a slight change in the microanatomical structure (1% -25%); + +: there are changes in the microanatomical structure (26% - 50%); + + +: occurred many changes in the microanatomical structure (51% -75%); + + + +: there are very many changes in the microanatomical structure (76% -100%).

Gill cellular conditions experiencing edema (Figure 2B), visible basement membrane began to stretch out, the field narrowing Lacuna cell deficiency causes gill function and difficulty in breathing process, so that the metabolism of the body began to fail. Edema is swelling of the cell or excessive accumulation of fluid in body tissues (Laksman 2003). The presence of edema can cause fusion of secondary lamella of the lamella. In this study, the occurrence of edema caused by the influx of Cd into the gills of *A. woodiana* resulted in the cell irritating so that the cell would swell.

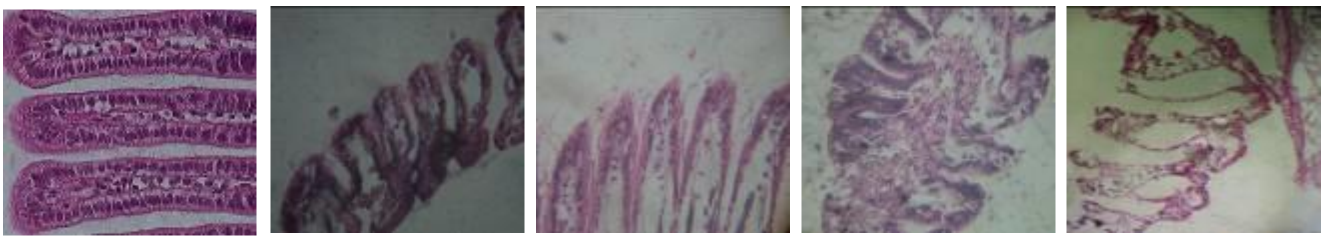
The process of entry of Cd into the gills by Palar (1994), together with other metal ions and the food that has been accumulated Cd, and will form ions that can dissolve in fat. Ions were able to penetrate the gill cell membrane, so they can get into the gills, and then there will be a process of loss of volume regulation in the cell. In this treatment were also seen pillar cells began to separate from the bottom of epithelial cells (middle lamella). When experiencing edema,l Cd accumulation in gills occurred at the accumulation of 0.5111 ppm.

Gills in Figure 2C has undergone thorough hyperplasia and the fusion is taking place in two parts of the middle lamella, with a marked by the epithelial cells started to scarp, accompanied by the loss widened Lacuna red blood cells and pillar cells apart. Laksman (2003) says that hyperplasia is a process of formation of excessive tissue due to the increase in cell volume. Hyperplasia caused by excessive edema so that red blood cells out of its capillary and separated from the backers. In the event, this hyperplasia Cd accumulation began at 0.6829 ppm exposure level.

Condition of cells and gill tissue had fused to lamella (Figure 2D), and began to show marked necrosis with epithelial cells in each lamella started together with epithelial cells on the other lamella, Lacuna also began to rupture causing respiratory function failure which affects the metabolism of *A. woodiana*. Secondary lamella fusion caused by the swelling in the cells of the gills (edema). The occurrence of secondary lamella fusion resulting in impaired function of the secondary lamella in the case of oxygen-making process and therefore contributes to the death of *A. woodiana* (Susilowati 2005). At a concentration of 5 ppm after 30 days *A. woodiana* experience death. In this incident, the gills accumulate heavy metals at a concentration of 0.9280 ppm.

At that last stage of a gill would experience the highest levels of damage, this damage can lead *A. woodiana* to experience the death of the level of necrosis and atrophy. Condition of cells and gill tissue necrosis and atrophy experienced (Figure 2E), characterized by the merging of each cell in lamella and lamella with bone loss starting institutions. Atrophy is a reduction (shrinking) the size of a cell, tissue, organ or body part (Harjono 1996). In this study occurred atrophy in primary lamella. Atrophy occurs due to experimental animals exposed to cadmium at high concentrations and in long exposure time. Cells in primary lamella shrinkage (atrophy).

Laksman (2003) states that the necrosis is cell's death that occurs due to hyperplasia and excessive fusion of secondary lamella, so that the gill tissue is no longer intact form or in other words, necrosis occurs accompanied with the death of a biota. In the event necrosis and atrophy of the accumulated Cd in the gills of *A. woodiana* started at 2.1279 ppm exposure and atrophy starting at the level of accumulation of 2.337 ppm.



A **B** **C** **D** **E**

Figure 2. Structural changes in gill cells *A. woodiana*. Note: A. Tues normal gills, B. Tues gill edema, C. Tues gill hyperplasia, D. Tues fusion gill lamella, E. Tues gill necrosis.

Microanatomical changes in kidney of A. woodiana *after exposure to Cd*

Changes in the microanatomical structure of the kidney of *A. woodiana* after administration of Cd are shown in Table 4. From this table, it is known that changes in cellular structure microanatomy kidneys began to occur at a concentration of 0.5 ppm for 7 days, edema of the tubules begin to appear and be perfect edema at 30 days and began to show more than 25% hyperplasia. Perfect hyperplasia is shown at a concentration of 1 ppm after 14 days of inspection, then the fusion epithelium of the kidney evenly shown at concentrations of 5 ppm after 30 days of inspection.

Table 4. Changes in cellular structure of kidney microanatomy *A. woodiana* after exposure to heavy metals cadmium with hematoxylin-eosin staining preparation.

Concentration (ppm)	Time of surgery (Days)	Edema	Hyperplasia	Fusion of lamella	Necrosis
0	7	-	-	-	-
	14	-	-	-	-
	30	-	-	-	-
0.5	7	+	-	-	-
	14	+++	-	-	-
	30	++++	++	-	-
1	7	+++	+	-	-
	14	++++	++++	++	-
	30	++++	++++	+++	-
5	7	++++	+++	++	-
	14	++++	++++	+++	+
(dead)	30	++++	++++	++++	+++
10	7	++++	+++	+++	-
	14	++++	++++	++++	+++
(dead)	30	++++	++++	++++	++++

Note: same as Table 9.

The kidney cells have shown complete necrosis at a concentration of 10 ppm after 30 days, while the concentration of 5 ppm to 10 ppm starting from day 14 and day of the 30th state of *A. woodiana* have been many who experienced the death (LC50), so the kidneys and gills partially preserved in a freezer with a temperature of -4 ° C for further examination. In Figure 2A, are shown in the picture kidney that is still in normal circumstances from the control *A. woodiana*.

Situation normal kidney cells and tissues in the control *A. woodiana* or the treatment of 0 ppm (Figure 3A), visible layer between cells in glomeruli and tubules and blood cells are still visible above and below normal. The metal accumulation in the kidney ranged from 0.0095 to 0.0242 ppm. Cd pollution levels, according to FAO (1972) still in the normal category below the threshold of fishery water quality (1 ppm), so it can be said that the content of Cd in the kidneys of *A. woodiana* the control is still normal. State accumulation is still at normal levels it may also occur due to *A. woodiana*, located among posterior kidney, heart, and pericardium (Suwignyo et al. 2005). Kidney position located on the inside and is relatively protected from the environment cause the accumulation of Cd is relatively small when compared to the accumulation of Cd in the gills.

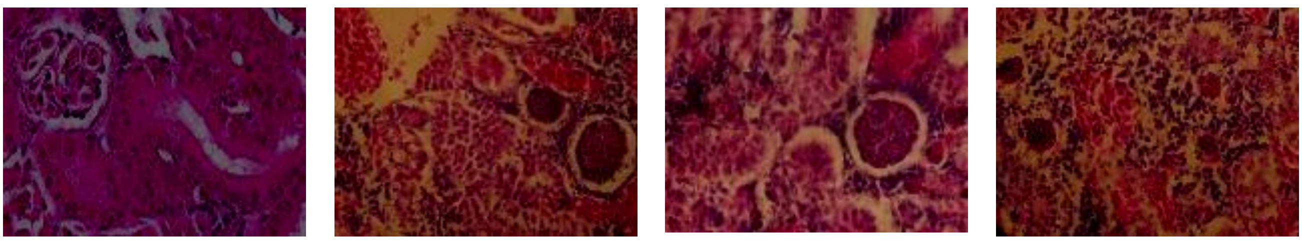
In Figure 3B, indicated changes in cell structure that have undergone kidney microanatomy edema in all parts of the tubules to glomeruli (indicated by black color), and seems to bleed blood cells due to accumulated Cd logan continuously. In clinical edema in kidney cells caused by erasification of proteins in the renal tubules in the network, so that the urine comes out containing excessive protein. In these conditions, the accumulation of Cd to the kidney began to be exposed at a concentration of 0.0200 ppm.

Then on further changes, where the higher Cd exposure then suffered kidney cell hyperplasia (Figure 3C), which is marked by the outbreak of the tubules, and the resulting mixing of intracell with extra fluid cell, and then also in the glomerulus looks very black, because the glomerulus has accumulated more Cd long, which will result in epithelial cells will rupture at any time. Then the blood cells were also seen indicating blackish blood has been contaminated with Cd. The range of Cd accumulation in kidney condition hyperplasia began to occur on exposure of 0.0849 ppm.

The highest level of damage to the kidney, necrosis of kidney cells have shown in Figure 3D, which has entered the stage of renal cell necrosis seen any broken tubules, glomeruli also broken so that mixed the cells with extra fluid cells, and whole blood cells were blackened due to Acute accumulation of Cd.

The content of Cd in kidney like this occurred at the exposure of 0.0786 ppm.

According to Atdjas (2008) accumulated Cd at the highest level will cause some kidney disorder that is poisoning the nephrons of the kidney (nephrotoxicity), proteinuria or protein in the form contained in the urine, diabetes where there is the content of glucose in the urine (glycosuria), and aminoaciduria or amino acid content in the urine accompanied by a decline in kidney filtration rate glumerolus.



A B C D

Figure 3. Structural changes in renal cell woodiana. Notes: A. Tues normal gills, B. Tues gill edema, C. Tues fusion gill lamella, D. Tues gill necrosis

CONCLUSION

There is significant heavy metal accumulation of Cd in each treatment against the gill and kidney *A. woodiana* as evidenced by the ANOVA test data for 475.3> 60150.3 0.000 and> 0.000 with an average significance level of 5% (P <0.05). There are structural changes in the kidneys marked by microanatomical forms which were edema, hyperplasia, fusion of lamella and necrosis, whereas in the kidney in proved by the occurrence of edema, hyperplasia, and necrosis of the tubules, glomeruli and mineralization in the blood cells to bleed.

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