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Study of the Impact of Heavy Metal (Cd, Pb and Hg) Contamination in Wedung Waters, Demak on *Anadara granosa* (Linnaeus, 1758)

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Abstract

Wedung Waters, Demak in Central Java Indonesia is a potential location for harvesting Blood Cockles, A*nadara granosa*. Anthropogenic activities such as agriculture and fishing have potential to cause the entry of heavy metals like Cd, Pb, and Hg into marine environment and affect the biota *A. granosa.* The test of Cd, Pb, and Hg contaminations in waters, sediments and *A. granosa* soft tissues have been performed in this study. Histopathology analysis in gill and hepatopancreas are done to see the impact of the contaminations in that biota. The result revealed that Cd, Pb and Hg are not detected in waters, but two of the pollutions, Cd and Pb, are found in sediment and *A. granosa* soft tissues. Cd and Pb present about 0.08 - 0,2 $mg.kg^{-1}$ and 3,35-6,46 $mg.kg^{-1}$ respectively in sediment Heavy metal test revealed that the Cd and Pb content in soft tissue of *A. granosa* present about 0,56-0,70 $mg.kg^{-1}$ and 0,05 -0,1 $mg.kg^{-1}$ respectively. We found inflammation in gills of *A. granosa* that can be seen from the infiltration of hemocyte, hyperplasia of goblet cell, gill swelling, hemocyte granulocyte, pigmentation, and cilia degeneration. In digestive gland we found the Lifting of epithelial cell, infiltration of hemocyte, pigmentation, necrosis of epithelial cell.

Keyword: *Anadara granosa, Heavy metal Cd,Pb,Hg, Histopathology*

INTRODUCTIONS

Pollution contamination such as heavy metals from anthropogenic activities into the marine environment has been reported to increase in recent decades [2]. Heavy metals are natural elements of waters and sea waters. Anthropogenic activities like Industrial, agricultural runoff (pesticides, fertilizer and herbicides), acid rain etc. contributed to increase metal content in sediment or marine environment [2;9;20].

Heavy metal pollutant is one of the biggest problems that pose a threat to the ecosystem. Heavy metals have the potential for bioaccumulation and biomagnification that cause heavier exposure to some organisms [11]. Heavy metals toxic is presisten because it has a tendency to accumulate in organisms and undergo food chain amplification, and the fact that they are not degraded make it even worse [38]. Accumulation of heavy metal in sediment can cause ecotoxicological effect in aquatic organism [14]. Heavy metal such Cd, Pb, and Hg have a high toxicity even in low doses and have the potential for biomagnification, bioaccumulation, and incorporation [45;40].

Bivalve is one of biota which is potential to be bio indicator of heavy metal contamination, especially for Cd and Pb [7]. *A. granosa* are recommended to be biomonitoring of heavy metal because of their capability to accumulate it. In Indonesia, *A. granosa* is a fairly commercial shellfish commodity. Production of shellfish in Indonesia from 2011 to 2015 increased by an average of 6.81% per year. In 2015, Indonesian shellfish production reached 59,613 tons where 85.26% of the number was from *A. granosa* [18]. Wedung is a sub-district in Demak Regency that contributes to the production of *A. granosa* species in Central Java Province of Indonesia [4].

Histopathology of gills and hepatopancreas is one of the tools used to view evidence of biomonitoring [17]. Some researchers see histopathological results on gills and hepatopancreas to be associated with the impact of heavy metal contamination on bivalves [32; 22; 37].

The aim of this study is to analyze Cd, Pb, and Hg in Wedung Waters, Demak in Central Java Indonesia and their effects on the bivalve of *A. granosa* by using histopathology analysis to the gills and digestive glands.

MATERIALS AND METHODS

The research was held in February and March 2019 at Wedung Waters, Demak Central Java Indonesia. The purposive sampling was done at four stations: Station A at 6°44'39.20"S - 110°33'4.37"E, Station B at 6°44'57.29"S - 110°33'17.02"E, Station C at 6°46'1.84"S - 110°32'21.60"E and Station D at 6°47'14.36"S - 110°33'25.79"E. The sampling locations are showed at Figure 1 below.

Figure 1. Sampling Location

Samples of 100 Blood Cockles catch using nets from each stations in February and March. The samples were stored in polyethylene plastic bags and put into a cool box. The temperature of the cool box were controlled to be below 4°C during transportation. Finally, the samples were stored in freezer with temperature of -40^{0}C before heavy metal analysis [20].

500 ml of sea water sampling was taken using a *Nansen* bottle on a surface layer of water and stored in polyethylene bottle [34]. HNO_3 was added into the sample to get sample with pH <2 [25]. Sediment samples were taken by following the procedure in [16] at a depth of 0 to 5 cm using a portable Ekman grab sampler.

Water Parameter Quality Measurement

Water parameter data such temperature, salinity, pH, and Dissolved Oxygen (DO) was taken directly /*in situ* during the study using Water Quality Checker [10;47]

Heavy Metal Analysis on Water and Sediment

Heavy metals Cd, Pb, and Hg in water are analyzed by following the procedure in Indonesian National Standard [25] for (Cd) test, [27] for (Pb) test [26], and for (Hg) test. The test was carried out by homogenizing 50 ml of sample water for the Cd test and 100 ml of sample water for the Pb test, with 5 ml of concentrated nitric acid. The water sample then heated up until its decreased to 15 ml in volume. Next step was adding *aquadest* into the samples until it reached 50 ml in volume for the Cd test and 100 ml in volume for Pb test. The solution then analyzed by Atomic Absorption Spectroscopy (AAS) graphite type with a wavelength 228,8 nm for Cd test and 283,3 nm for Pb test.

The Hg analysis procedure for water was carried out by homogenizing 100 ml of water with H_2SO_4 and 2.5 ml of HNO_3. 15 ml of $KMnO_4$ solution was then added to the sample and waited for 15 minutes before adding 8 ml of $K_2S_2O_8$. Samples were heated in a hot plate for 2 hours at 95°C before adding hydroxylamine-NaCl to reduce $KMnO_4$. The final step was adding 5 ml of $SnCl_2$ before analyzing it using AAS hydric with a wavelength of 253.7 nm.

Grinded sediment sample using 100 meshes digested by weighing 0.1 g of a dry sample weighed beforehand carried out at around 170°C for 24 hours using a mixture of 2 ml of HNO_3, 2 ml H_2O_2 and 0.5 HF. After digestion, the solution filtrated and diluted to final volume of 25 ml to measured using ICP OES with wavelengths of 214,439 nm for Cd, 220,353nm for Pb and 184,887nm for Hg [33]

Heavy Metal Analysis on Blood Cockle

Analysis of Cd and Pb on Blood Cockle refers to the Indonesian National Standard [23]. Amount of 5 grams of soft tissue of Blood Cockle being destructed at 450^{0}C with a furnace for 18 hours. after that add 1 ml of 65% HNO_3 then evaporated on a hot plate until dry, sample destroyed again at 450^{0}C for 3 hours, then added 5 ml of HCl 6 M and heated with a hot plate. After heating 10 ml of 0.1 M HNO_3 adding until the volume reaches 50 ml. Samples were analyzed by AAS with a wavelength, for Cd 228.8 nm, while Pb at 283.3 nm.

Hg test was carried out referring to the Indonesian National Standard SNI [24], a number of 5 grams of sample was added 3 pieces of boiling stone, then added 10 mg - 20 mg V_2O_5 and given 10 mL HNO_3 65% and 10 mL H_2SO_4 95% - respectively 97%. The sample is then heated, then rinsed with 15 mL deionized water, 2 drops of 30% H_2O_2 and finally added with distilled water to a volume of 100 ml. The heavy metal Hg in the sample was read using AAS with a wavelength of 253.7 nm.

Histopathology Analysis

A. granosa was fixation using Davidson's solution then dehydrated with 70-95% and 100% ethanol, then planted using paraffin. The sample is then cut using a microtome. Furthermore, it was stained using Hematoxylin and Eosin. Histopathological results were read using a microscope with a magnification of 10x-1000x. [6].

RESULT AND DISCUSSION

Water Parameter

The temperature at the four station ranges from 28.8°C - 31.6 $^{\circ}$C which is common temperature in tropical waters. DO generally increases with temperature, DO ranges between 5.05- 5.4 mg l^{-1}. pH ranges between 8 - 8.55 that make it possible for *A. granosa* to live. Salinity ranges between 16.70 - 29.11 ppt that is within the *A.granosa* habitat range [3]. Result of water parameter shown at Table 1.

Table 1. Water Parameter

Parameter	Station				Month
	A	B	C	D	
Temperature (^{0}C)	29,2 ± 0,14	29,35 ± 0,64	30,4 ± 0,00	31,55 ± 0,49	February
pH	8 ± 0,00	8,30 ± 0,14	8,00 ± 0	8,2 ± 0,14	
Salinity (ppt)	18,9 ± 0,14	18,15± 0,02	29,11 ± 1,12	16,70 ± 0,79	
DO (mg.l^{-1})	5,19 ± 0,01	5,40 ± 0,30	5,10 ± 0,13	5,06 ± 0,27	
Temperature (^{0}C)	28,8 ± 0,00	28,95 ± 0,07	29,85 ± 0,07	31,6 ± 0,14	March
pH	8,15 ±0,07	8,2 ± 0,00	8,55 ± 0,07	8,3 ± 0,00	
Salinity (ppt)	20 ± 0,16	18.56 ± 0,002	26,445 ± 0,49	17,265 ± 1,75	
DO (mg.l^{-1})	5,3 ± 0,21	5,135 ± 0,02	5,055 ± 0,09	5,24 ± 0,37	

Heavy Metal Test Results for Water and Sediments

Heavy metal analysis of Cd, Pb, and Hg were not detected in seawater from four stations in Wedung Waters, Demak. The results showed that the lowest content of Cd in February was found at station C with a value of 0.08 mg.kg^{-1} and the highest value at station D with a value of 0.11 mg.kg^{-1}. While for sample that were taken in March 2019, the lowest value of the Cd found at station D with a value of 0.11 mg.kg^{-1} and the highest at Station B and C with a value of 0.20 mg.kg^{-1}. The lowest Pb content in February was found at Station A with a value of 3.35 mg.kg^{-1} and the highest value at station D with a value of 5.38 mg.kg^{-1}. The Pb content value in March was higher as compared to the previous month, with the lowest grade (Pb) value at station A of 4.77 mg.kg^{-1} and the highest value at Station C with a value of 6.46 mg.kg^{-1}. Hg content were not found at all stations both in February and March 2019. The complete test results are provided in Table 2.

Table 2. Content of Heavy Metals in Sediments

Parameter	**Heavy Metals content in Sedimen at Station (mg.kg^{-1})**				**Target Level (mg.kg^{-1})***	**Month**
	A	**B**	**C**	**D**		
Cd	0,10 ±0,01	0,10 ±0	0,08 ±0	0,11 ±0	0.8	
Pb	3,35 ±0,05	3,90 ±0,04	3,36 ±0,04	5,38 ±0,02	85	February
Hg	Nd	Nd	Nd	Nd	0.03	
Cd	0.17 ±0.01	0.20 ±0.02	0.20 ±0.01	0.11 ±0.01	0.8	
Pb	4.77 ±0.09	6.34 ±0.21	6.46 ±0.09	5.74 ±0.24	85	March
Hg	Nd	Nd	Nd	Nd	0.03	

Source: Primary Data
*IADC (*International Association of Drilling Contractors*) / CEDA *Central Dredging Association*) (1997)
Nd = Not detected

The Kruskal Wallis test of Cd content in sediments from four stations A, B, C, and D showed a *p-value* of 0.072 in February and 0.106 in March (> 0.05). Therefore, there was no significant difference of Cd content in sediment taken from the four stations. The Kruskal Wallis test for Pb levels in sediments resulted with *p-value* of 0.112 in February and 0.104 in March (> 0.05). That means there was no significant difference of Pb content in sediment taken from the four stations.

Hg content was not detected in the water and sediments. This indicates that the Wedung Waters are not polluted by Hg. Cd and Pb content were also not detected in the water but were detected in sediments. Cd and Pb content of sediments are below the threshold set by the CEDA [15] which is 0.8 mg.kg^{-1} for Cd and 85 mg.kg^{-1} for Pb. This means that Pb and Cd content in sediments in the Wedung Waters are not too dangerous for the environment.

By looking at the activities in Wedung, it is suspected that the contamination of Cd and Pb to marine environment comes from anthropogenic activities in the form of agriculture and vessel activities. Referring to the Statistics Data of the Wedung District (2015), the main livelihoods of the residents dominated by farmers with a total land area of 7.000,40 ha and fishermen with a total of 1,228 units of vessels. Agricultural activities in the form of the use of fertilizers, pesticides, and herbicides cause the contamination of Cd and Pb to the waters. [12] stated the presence of Cd and Pb content used in fertilizers and pesticides. This statement was confirmed by [21] and [39] stating that pesticides and herbicides contain lead and arsenic which contaminate the sea and accumulate in sediments and bivalves. Pollutants from antifouling ship paint and ship fuel are associated with Cd and Pb metal contamination [48;5]. Cd and Pb are not detected in water but present in sediments because sediment has the ability to absorb heavy metals better than the water [13]. [41] further stated that much more a little of 1% metal contaminants (and other pollutants) are dissolved in water, while more than 99% are stored in sediments. Heavy metals contained in the waters are then absorbed in particles and accumulated in sediments.

Heavy Metal Test Results on Blood Cockle Soft Tissue

The results of heavy metal content in soft tissue showed that Hg was not detected in soft tissue shells. Samples taken in February showed that the lowest Cd content was found at Station C with a value of 0.60 mg kg^{-1} and the highest was at Station D with a value of 0.66 mg kg^{-1}. Observations taken in March showed the lowest Cd level found at station B with a value of 0.56 mg kg^{-1} and the highest was at station C with a value of 0.70 mg kg^{-1}. From the sample taken in in February showed that the lowest Pb content was found at station C with a value of 0.05 mg / kg and the highest was at station D with a value of 0.1 mg kg^{-1}. In March showed the lowest Pb level found at station D with a value of 0.06 mg kg^{-1} and the highest was at station A and B with a value of 0.09 mg kg^{-1} Cd, Pb and Hg test results from soft tissue Blood Cockle are provided in Table 3.

Table 3. Heavy Metal Content of *A.granosa* Soft Tissue

Parameter	**Heavy Metal Content of *Anadara granosa* Soft Tissue at Station (mg.kg^{-1})**				**Standard SNI* & EC 2006** (mg.kg^{-1})**	**Month**
	A	**B**	**C**	**D**		
Cd	0,64 ±0,18	0,64 ±0,20	0,60 ± 0,02	0.66 ± 0.08	1	February
Pb	0,08 ±0.03	0,08 ±0,02	0,05 ±0	0,10 ±0,05	1,5	
Hg	Nd	Nd	Nd	Nd	1	
Cd	0,62 ±0,05	0,56 ±0,01	0,70 ±0,06	0,66 ±0,13	1	March
Pb	0,09 ±0,03	0,09 ±0,03	0,08 ±0,04	0,06 ±0,01	1,5	
Hg	Nd	Nd	Nd	Nd	1	

*SNI (National Indonesian Standard number SNI 7387: 2009 **EC Commission Regulation 2006

The results of the Kruskal Walis test on the Cd content from the soft tissue of Blood Cockle at the four stations showed there was no difference in Cd content from four stations with *p-value* of 0.983 in February and 0.421 in March (>0.05). Kruskal Walis test for Pb levels at the four stations gave the *p-value* of 0.435 in February and 0.487 in March (> 0.05) which means that there were no significant differences of Pb content in the four stations.

The value of Cd and Pb content in *A. granosa* soft tissue are still below the maximum level of Cd and Pb content in Indonesian Nasional Standard [28] and the European Union through EC (2006) [8]. Therefore, *A. granosa* from the Wedung Waters are still safe for consumption. Heavy metals enter the marine invertebrates body generally through a solution and food [35]. Heavy metal uptake involves free metal ions such as Cd^{++} and Zn^{++} as bioavailable dissolved forms, although at equilibrium free metal ions at sea, often only represent a small percentage of the total concentration of dissolved metals [50;43;29]. Absorption of heavy metals into the vertebral body can occur passively through diffusion or with permeable surfaces. Scenarios that allow for passive absorption through a diffusion process are that heavy metals will bind intracellularly to metal-binding ligands [49;44;36].

Histopathology test results

Histopathological observations on the Gills of Blood Cockle in February showed the presence of inflammation as indicated by the presence of hyperplasia cell goblet, cilia degeneration, hemocyte infiltration and pigmentation and the presence of gill swelling (Figure 2). Histopathological observations of gills in March also showed the same results. Gills are known as one of the organs that are most affected in many types of toxicity as it become entrances of toxins dissolved in water [51]. *Cladophora glomerata* contaminated by Cd^{++} showed experienced changes in gills such as curling and secondary lamella fusion, epithelial hypertrophy, epithelial hyperplasia, pillar cell damage, edema, swelling, aneurysm, necrosis, and increased mucus secretion. This condition is getting worse as the dose and exposure time increased [31]. Kahn [19] stating the existence of histopathological in *Anodonta cygnea* due to heavy metal contamination with a sequence of levels of metals in soft tissue follows the order of Fe> Zn> Mn> Pb> Cu> Cr> Ni> Cd, indicating an abnormality in the gills such as flat fusion, widening of the hemolymphatic sinus, degeneration of the cilia, hemocyte infiltration, and gill swelling.

The pathological effects of accumulated Cd are thought to negatively interfere with the transport of oxygen needed for respiration by gills [30]. Degeneration of the gills, in general, results in an unbalanced oxygen delivery, which can trigger functional problems in other metabolic organs such as the liver and kidneys [51]. Amin [1] stated the ciliary degeneration in *P. viridis* due to exposure to heavy metals such as Pb that entered during the process of taking food and food diffusion caused a decrease in the growth rate and caused death.

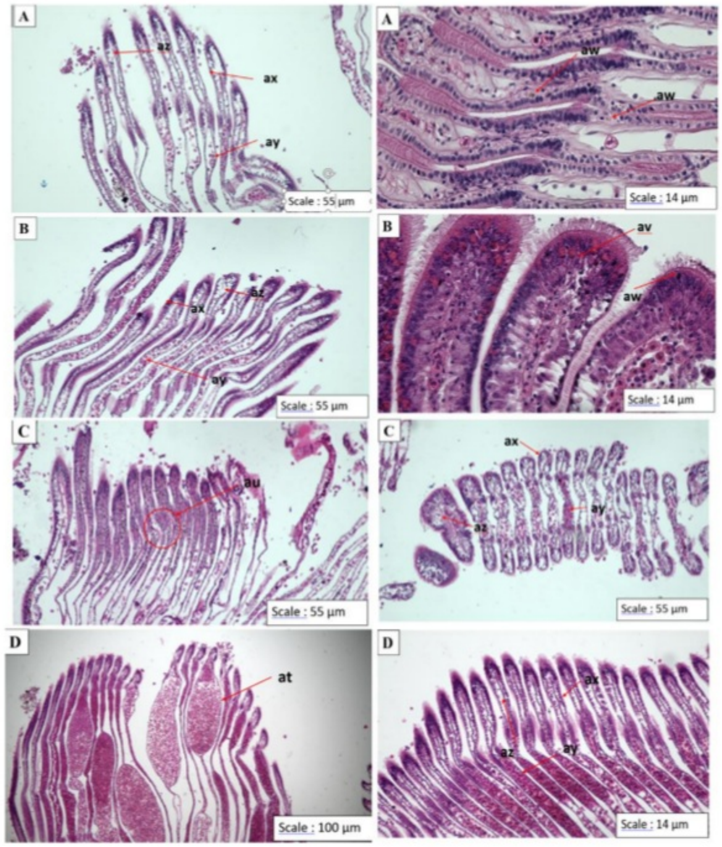

Figure 2. Gill histopathology of *A. granosa* (A) Station A, (B) Station B, (C) Station C and (D) Station D.(az) Hyperplasia goblet cell, (ay) Hemocyte infiltration (ax) Cilia degeneration,(aw) Pigmentation, (av)Hemocyte granulocyte,(at) Gill swelling,(au) Lamella fussion.

Histopathological observation of the digestive glands, namely hepatopancreas in four samples also showed an indication of inflammation, it was seen the presence of hemocyte infiltration, pigmentation and necrotic epithelial cells and epithelial cell flattening (Figure. 3). Khan [19] also found similar histopathological effects of Pb and Cd from contaminated areas on *Anodonta cygnea* (Linea, 1876) in the form of inflammation, hydropic vacuoles, and lipofuscin pigments. Shaw and Handy [42] found that *Mytilus edulis* exposed to Cd with concentration of 20 μg / l and 50 μg / l, the highest concentration of Cd presents with sequence of the digestive glands, gills, and then posterior adductor muscle. Cd causes pathology in the digestive gland, especially at a dose of 20 μg / l which causes inflammation in the form of hemocyte infiltration.

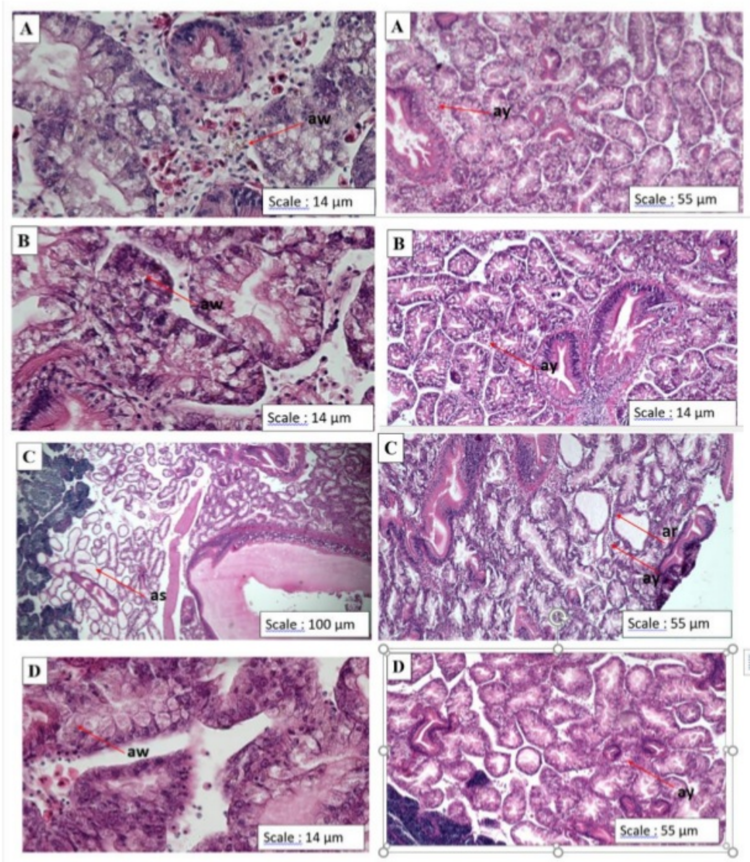

Figure 3. Hepatopancreas histopathology of *A. granosa* A) Station A, (B) Station B, (C) Station C and (D) Station D (ay) Hemocyte infiltration, (aw) Pigmentation, (as) Flattening epithelium cells, (ar) Necrotic epithelium cells

Conclusions

The waters of Wedung Demak are not contaminated by Hg metal. However, Pb and Cd are found in the sediments and soft tissue of *A. granosa*. Cd content in sediment ranges from 0.08 to 0.2 mg. Kg^{-1} while Pb ranges from 3.35 to 6.46 mg. Kg^{-1}. The value is still not too dangerous for the environment. In soft tissue of Blood Cockle, Cd found around 0.56 - 0.7 $mg.kg^{-1}$ and Pb of 0.05 - 0.1 $mg.kg^{-1}$ which is still safe for consumption. Histopathology test result indicate inflammation of the gills and hepatopancreas.

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